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AUDITION

1 MEDIAL GENICULATE NEURONS OF THE UNANESTHETIZED SQUIRREL MONKEY: PROCESSING OF SIMPLE AND COMPLEX ACOUSTIC STIMULI.
G. E. Alexander, D. Symmes and J. D. Newman. BBBr., NICHD-NIH, Bethesda, Md. 20014.

The activity of single neurons was recorded from the main subdivisions of the medial geniculate nucleus of awake unanesthetized squirrel monkeys (Saimiri sciureus). The sample was composed of 269 cells tested with a variety of acoustic stimuli. Sustained increase in spike discharge was the most frequently encountered response pattern to tone bursts, noise bursts, and taped Saimiri vocalizations. Approximately 98% of the neurons tested responded to clicks, 92% to broad band noise bursts, and 91% to tone bursts in the range between 0.5 and 21 KHz. Sixty percent of the units were driven most effectively when the stimuli were presented binaurally with matched intensity, 29% were driven best by contralateral stimulation, and the remaining 11% were most sensitive to ipsilateral stimuli. About 25% of the cells were characterized by a variety of non-monotonic spike vs. intensity curves, including transitions from activation to suppression of firing with increasing stimulus intensity. In the other 75% of cells which had monotonic spike vs. intensity curves, the relationship between firing rate and stimulus intensity was in each case best described by a power function. In the ventral subdivision of the medial geniculate approximately 90% of the cells had definable best or center frequencies, whereas this was true of only 30% of cells in the magnocellular subdivision.

Responses of individual medial geniculate neurons to tape recorded Saimiri vocalizations were predictable from each cell's response to artificial stimuli, in terms of frequency vs. intensity tuning curves, spike vs. intensity functions, and binaural interactions. In comparison to neurons in the primary and secondary auditory cortices, medial geniculate cells showed less selectivity in their responsiveness to a standard set of species-specific vocalizations.

2 THALAMOCORTICAL, CORTICOTHALAMIC AND CORTICOTECTAL PROJECTIONS TO AND FROM PHYSIOLOGICALLY IDENTIFIED LOCI WITHIN THE AUDITORY CORTICAL FIELDS AAF, AII AND AI. R.A. Andersen*, H. Patterson*, P. Knight*, B. Crandall* and M.M. Merzenich* (Spon. R. Nicoll). Coleman Lab., Univ. of Calif., San Francisco, Ca. 94143.

We entertain the possibility that different cortical fields encode or represent different aspects of auditory sensation. We have studied the thalamocortical, corticothalamic and corticocollicular connections of 3 of these auditory cortical fields in the cat, the anterior auditory field (AAF), AI and AII. After initially mapping the representation of the cochlea over a part of one or more of these fields using microelectrode recording techniques, tritiated leucine and/or horseradish peroxidase were injected at physiologically defined loci. Thalamocortical and corticothalamic projections to and from restricted AAF and AII loci were found to be purely ipsilateral and precisely reciprocal. A similar reciprocity has been previously described for the complex connections of AI and the medial geniculate body (MGB) (Colwell and Merzenich, Anat. Rec. 181:336, 1975), suggesting that such thalamocortical-corticothalmic reciprocity is the rule for auditory cortical fields. The principal reciprocal projection of a restricted AAF locus is to and from a column of neurons extending throughout the deep dorsal division of MGB. This reciprocal projection continues into the posterior group, where it breaks into several discrete columns, and into the caudal MGB, where it extends out to the lateral margin. AAF-deep dorsal MGB connections are ordered, consistent with a basal-to-apical cochlear representation within the lateral-to-medial dimension of the deep dorsal MGB. There was a less dense but restricted reciprocal projection to the rostrolateral part of MGB. With AII injections, a complex and diffuse reciprocal array restricted to the caudal MGB was observed.

Neurons at any AI locus projected to the caudal region of the inferior colliculus (IC), where their terminals formed restricted sheets within the pericentral and the central nuclei. A physiologically identified sector of the cochlear partition represented within AI always projected bilaterally to the same cochlear place represented within the two central nuclei. The corticotectal projection from an AII locus formed a layer of grains within the pericentral nucleus. No projections from AAF to IC have been observed.

Comparisons of the projections of these 3 fields onto the thalamus and IC show structural similarities suggesting certain developmental as well as functional commonalities. However, the overall connectional organization of each cortical field is unique, implying functional segregation and parallel processing of information ultimately giving rise to various aspects of auditory sensation. Supported by NIH Grant NS-10414.

3 **FORWARD AND BACKWARD MASKING OF CLICKS AND BRAINSTEM EVOKED RESPONSE CORRELATES.** **Rüdiger D.Brinkmann*,and Michael Scherg* (SPON: E.Pöppel). Max Planck Institut für Psychiatrie,8000 München 40 Fed.Rep.Germany**

The superior olive is the first waystation of the auditory system,where inputs of the two ears converge onto the same neurons and comparisons of differences in time of arrival and intensity are performed for the first time. Since wave III of the auditory brainstem evoked response has been related to the superior olive,we were interested to see,whether this wave contributes to our understanding of temporal masking.Under conditions of sequential dichotic presentation the masking click was held constant at 55 dB SL,while the probe click was presented with 55,45,35,25,and 15 dB SL either leading (backward masking) or lagging (forward masking). In the psychophysical part of the experiment subjects had to respond to the probe click as soon as it could be heard. The subjects then entered the electrophysiological part of the experiment. They were exposed to the combinations of intensities as mentioned above and the clicks were temporally separated according to the psychophysical findings. The brainstem evoked responses correspond closely to the stimulus conditions applied,in the sense,that a superimposition of the two brainstem evoked responses can be demonstrated. The latencies of the observed positive deflections coincide with the expected peaks due to each of the two clicks. Moreover,the temporal interval necessary for the detection of the probe click can be predicted from the latency of wave III for each of the conditions under investigation. This temporal interval (Δt) is a function of the difference between the latencies of wave III of the probe and the masking click ($LatIII_P - LatIII_M$) plus a constant time interval (C) of 1.4 ms in forward masking and .7 ms in backward masking with respect to the given intensities. in general Δt can be described by the following model: $\Delta t = f(LatIII_P - LatIII_M) + C$

This model allows to evaluate the consistency of the psychophysical judgements of subjects engaged in a temporal masking task. The different values for 'C' in forward and backward masking are in good accordance with reports on comparable psychophysical data.

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4 TRAVELING WAVE PARAMETERS IN THE KITTEN COCHLEA ESTIMATED FROM RESPONSE PROPERTIES OF NEURONS IN ANTEROVENTRAL COCHLEAR NUCLEUS. John F. Brugge, Eric Javel and Leonard M. Kitzes*. Dept. Neurophys. and Waisman Cntr., Univ. Wisconsin, Madison, WI 53706

Response areas, which plot discharge rate vs stimulus frequency at various SPLs, were obtained from AVCN cells of kittens during the first month postpartum. They are compared with similar data from AVCN of adult cat (Gibson et al, Psychophysics and Physiology of Hearing) and auditory nerve of adult monkey (Anderson et al, J. Acoust. Soc. Am. 49:1131). Most cells recorded in kitten AVCN had characteristics frequencies (CF) below 4 kHz, indicating that they were excited by basilar membrane motion primarily in the apical half of the cochlea, a region which may undergo structural changes during this time. After the first postnatal week the shapes of the response areas closely resemble those of response areas of auditory nerve fibers and AVCN neurons having the same CF in the adult. For CF below 1 kHz the response area is skewed toward high frequencies; around 1 kHz the curves become symmetrical; at higher CF there is an abrupt high-frequency cut off and shift of the curves toward lower frequencies. Measurements of the frequency interval between CF and high-frequency cut off, at 20 dB above threshold, agree well with adult values. Intervals were converted to distance along the basilar membrane using Greenwood's function relating distance to frequency. This distance may be taken as an estimate of the length of the apical segment of the traveling wave envelope at various locations along the basilar membrane given by the neuron's CF. Results indicate that in kittens as young as 6 days of age the basilar membrane supports a traveling wave along most of its length and that the dimension of the apical segment is similar to that in the adult cochlea. At the cochlear apex this length is about 3 mm; it decreases to a somewhat constant average value, less than 0.5 mm, in the basal half of the cochlea. During the first postnatal week, AVCN neurons are also responsive within a restricted frequency-intensity domain. However, the curves are broader than those in older animals and may spread for considerable distances in the high frequency direction. Period histograms were constructed for AVCN cells whose discharges are locked to cycles of low frequency tones. From the histograms a straight line phase-vs-frequency plot is constructed. The slope of the line is a time delay from which can be determined mechanical travel time along the basilar membrane. Cochlear propagation time in kittens in the third and fourth postnatal weeks approaches that in adults at all CFs. Travel time appears slightly lengthened in animals younger than two weeks of age. While mechanical properties of the kitten cochlea mature at an early age, many response properties of AVCN neurons are delayed for several weeks after birth. NIH Grants NS12732, HD03352.

5 SOME ASCENDING PROJECTIONS OF THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY IN CAT. Judy Brunso-Bechtold, K. K. Glendenning, and R. B. Masterton. Dept. Psych., Florida State University, Tallahassee, Fla. 32306.

Because the cells of the medial nucleus of the trapezoid body (MTB) are scattered among the fibers of the trapezoid body, its projections have been difficult to study. Nevertheless, MTB projections to the ipsilateral lateral superior olive have been deduced with degeneration techniques. The present investigation confirms this deduction by showing that small electrophoretic injections of HRP in the lateral superior olive result in a large number of backfilled neurons in MTB.

However, the projections of MTB are not restricted to bulbar structures. Following electrophoretic injections of HRP in the nuclei of the lateral lemniscus, a significant projection from the ipsilateral MTB is found. Furthermore, occasional backfilled neurons in ipsilateral MTB are found after small injections of HRP in the central nucleus of the inferior colliculus. Thus, MTB in addition to being part of the LSO system, is also part of a separate system that ascends to pontine and midbrain levels. (Supported by NIH:NS-7726)

6 GLYCINE EFFECTS ON RESPONSE PATTERNS OF NEURONS IN THE COCHLEAR NUCLEI. D.M. Caspary and D.C. Havey*. Division of Neurobiology, Department of Medical Sciences, Southern Illinois University School of Medicine, Springfield, IL 62704.

Glycine has been found in varied concentrations in different regions of the cochlear nuclei (Godfrey et al., J. Histochem. Cytochem., in press, 1977). Godfrey et al. reported that glycine levels in the cochlear nuclei (CN) are similar to those reported for spinal cord gray matter, where evidence exists for its role as a possible inhibitory transmitter (Aprison et al., Handbook of Neurochemistry, 1970, Vol. 3). The present study examined changes in both auditory evoked and spontaneous single unit activity in response to iontophoretic application of glycine. All neurons were studied at their best frequency, 20-30dB above the unit's threshold. A summating or current balancing channel, or a separate current barrel was used in all experiments. Post-stimulus time histograms (PSTH's) and interspike interval histograms (ISIH's) were obtained before, during, and after application of glycine onto CN neurons of the anesthetized chinchilla. Selective effects of glycine were observed on area-specific response patterns and spontaneous activity. Pauser and build-up type neurons from the dorsal cochlear nucleus (DCN) occasionally exhibit increased latency of onset response in PSTH's with iontophoretic application of small amounts (0.5-50 nA) of glycine (0.5M). Other pauser and build-up neurons exhibited a generalized inhibition of driven and spontaneous activity. Primary-like neurons typical of ventral cochlear nucleus (VCN) did not show a specific effect on the initial portion of the response. Glycine-responsive neurons showed either generalized depression of activity, or a striking inhibition of spontaneous activity with little reduction of driven activity. Frequently, glycine application caused total cessation of all spontaneous activity even in neurons with moderate to high rates of control spontaneous activity. Increasing doses of glycine progressively increased the selective effect in all glycine-sensitive neurons examined. In certain VCN neurons, increased glycine resulted in a response decrement resembling the reduction in response associated with a decrease in stimulus intensity.

Preliminary findings indicate that GABA also has an inhibitory effect on some CN neurons, but seems less effective than glycine. Further studies of the glycine and GABA effects on area-specific response patterns may suggest relationships with a specific cell type, i.e., small cells or interneurons, or neurons receiving descending inputs.

7 AUDITORY NERVE PROJECTIONS TO COCHLEAR NUCLEUS DEMONSTRATED BY ANTEROGRADE TRANSPORT METHODS. J.H. Casseday and D.R. Jones*, Depts. Surgery and Psychology, Duke University, Durham, NC 27710

To investigate the possibility that projections of the auditory nerve could be studied by methods utilizing anterograde axonal transport, tritiated leucine (250-1500 μCi) was injected through the round window into the cochlea of cats. After a survival period of 6-7 days, standard autoradiographic methods were used to show transport of the labeled amino acid to the cochlear nucleus. The result showed that variations in overall density of silver grains in the cochlear nucleus were consistent with recent reports on the projection pattern of the auditory nerve obtained by degeneration methods. For example, the density of labeled fibers was greatest in the ventral cochlear nucleus and least in the dorsal nucleus, where only a few labeled fibers were found in the molecular layer.

The most interesting finding concerned variations in the proximity and density of silver grains around cell bodies within each subdivision of the cochlear nucleus. These variations suggested marked differences in the innervation pattern of different cell types. In general cells of similar morphology had similar patterns of silver grains around their somas. For example in the anterior part of the anteroventral cochlear nucleus, spherical cells were most often totally surrounded by silver grains; whereas in both anteroventral and posteroventral cochlear nucleus, fusiform cells were never surrounded by grains, and when present, the grains were clustered at the poles of the cell. In the central area of the posteroventral nucleus the soma and proximal dendrites of octopus cells were encrusted with silver grains. The pattern of label around an octopus cell is shown in darkfield and lightfield illumination in Figs. 1 and 2 respectively. (Supported by NIH grant NS 12322)

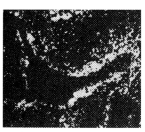


Figure 1.

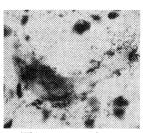


Figure 2.

8 ONGOING HAIR CELL PRODUCTION, MATURATION, AND DEGENERATION IN THE SHARK EAR. Jeffrey T. Corwin, Neurobiol. Unit, Scripps Inst. Oceanography, and Dept. Neurosciences, Sch. Med., Univ. Calif., San Diego, La Jolla, CA 92093.

In carcharhinid sharks the macula neglecta is an auditory epithelium which contains thousands of aligned hair cells and forms part of the lining of the posterior canal duct. It is a highly differentiated structure, but inspite of this, surface counts of its hair cells indicate that great numbers are added post-embryonically.

The origins of these cells were investigated by pulse injection of juvenile sharks with 3H-thymidine. In animals with the shortest survival time autoradiography demonstrated the label within the nuclei of relatively undifferentiated basal cells in a stratified marginal epithelium which forms a nonsensory zone extending 250 μm outside the edge of the sensory epithelium. With longer survival times the label progressed into the surface strata within this marginal epithelium and eventually entered the hair cell population in the peripheral sensory epithelium. While in the stratified marginal epithelium some of the cells develop certain cytological characteristics of hair cells. At this time they also develop associations with neurites even though they may be 200 μm peripheral to the edge of the sensory epithelium.

In correspondence with the above, scanning electron microscopy of ears from juvenile and mature adults has shown a gradation of hair cell surface form from the periphery to the center in each sensory epithelium examined. Peripheral cells have small surface outlines, short stereocilia, and a kinocilium which in some cells is shorter than normal. However, these cells possess a full complement of cilia arranged in a miniature version of the geometrical array found in central hair cells. There is a gradual change in these characteristics as one progresses inward to the central portion of the epithelium which is mainly composed of large, mature hair cells. In the adult ears in addition to the large cells a third type of hair cell was sparsely distributed in the central region. The small size, the lack of geometrical form, and the reduced number of cilia of this third cell type suggest that they may be senile or degenerating.

These results clearly show that the possibility of post-embryonic hair cell addition and turnover should not be ruled out simply on the basis of the high structural differentiation found in epithelia of many acousticolateralis organs.

(Supported by grants to Dr. T.H. Bullock from NSF and NIH and grants to Mid-Pacific Marine Laboratory from ERDA.)

9 ROLE OF NEOCORTEX IN INTERAURAL INTENSITY AND PHASE-ANGLE DISCRIMINATION: DETECTION VS. IDENTIFICATION. Jerry Cranford, Makoto Igarashi, Ronald DeWitt,* Dept. Otorhinolaryngology & Communicative Sciences, Baylor College of Medicine, Houston, Texas, 77030

At the present time, a major issue concerning the effects of auditory decortication on sound localization is whether or not operated animals retain a normal capacity for discriminating the small interaural differences in arrival time (phase) or intensity which result from the spatial separation of sound sources relative to the head. Heffner & Masterton (1975) have suggested that the deficit seen with cortical lesions, rather than reflecting a disruption of the animal's normal perception of the location of sounds, may be the result of an "auditomotor or associative" deficit involving the animal's inability to act appropriately on the basis of its normal perception. Neff & Casseday (1977), on the other hand, believe that the auditory cortex is necessary to allow animals to recognize that the stimulus to one ear differs in some respect (intensity, time of arrival, sequential arrangement of sounds) from the stimulus to the opposite ear.

The present experiment was designed to provide data relevant to this controversy. Using earphones and an active avoidance training procedure, the effects of auditory cortex ablations on the cats' ability to detect reversals in the interaural phase-angle or intensity relations of binaural 1-kHz tones were studied with seven cats. For both auditory decorticate (ablation of AI, AII, Ep, SII, I-T) and unoperated cats the detection thresholds for interaural intensity and phase-angle were found to be close to 1-dB and 5^{o} respectively. In addition, we found that both operated and unoperated cats transferred at above chance levels from the original lateralization task involving the detection of interaural reversals of phase-angle or intensity to a new paradigm which required the cats to identify, in an absolute sense, which ear received the leading or louder signals. Thus, the present investigation provides additional evidence that the neocortex has no primary sensory role in sound localization.

H. Heffner & B. Masterton, J. Neurophysiol. 38(1975) 1340.
W. D. Neff & J. H. Casseday, J. Neurophysiol. 40(1977) 44.
Supported by NINCDS grants NS11812 and NS10940.

10 QUANTITATIVE ANALYSIS OF CLICK ELICITED RESPONSES IN ANTEROVENTRAL COCHLEAR NUCLEUS NEURONS. John W. Dickson, Mario A. Ruggero and Robert E. Wickesberg*. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

We have studied the post-stimulus time histograms of the responses to clicks of 111 anteroventral cochlear nucleus (AVCN) neurons in barbiturate-anesthetized cats. Most units were stimulated with clicks of both polarities at a variety of intensities; characteristic frequencies (CF's) were determined for 93 units. As in the cochlear nerve (N. VIII), the click-evoked histograms from low-CF units were multimodal. The "time" of each peak was defined as the arithmetic mean of the times of the spikes in the peak. Using statistical and graphical methods, we studied the relationship of mean interpeak interval to CF, the interdigitation of the peaks elicited by the two polarities, the consistency of the mean interpeak intervals measured at different polarities and intensities, and peak latency shifts with intensity changes. Most of our results confirm the similarity of AVCN and N. VIII click responses. Results not previously reported for click responses in AVCN include occasional systematic changes in interpeak interval within a single histogram and, for low-CF units, similarity of peak times for units of similar CF. The former, seen most often at high intensities, is reminiscent of nonlinear effects seen in the basilar membrane and in N. VIII. The latter suggests a uniformity of AVCN responses which may be important for processing of temporally encoded signals by higher centers (e.g. for sensing interaural phase differences). (Supported by N.I.H. grants NS12732, NS02024, NS51561 and a NSF Graduate Fellowship (R.E.W)).

11 COCHLEAR NUCLEUS UNIT RESPONSES IN CONSCIOUS RABBITS. John F. Disterhoft, Scott Evans* and Leslie Sargent Jones*. Dept. Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Responses of single neurons in the cochlear nucleus of young adult male albino rabbits to pure tones were characterized. The rabbit's head was fixed to a stereotaxic by 4 restraining bolts. A visual approach to the cochlear nucleus was achieved by opening the foramen magnum and removing enough of the occipital bone to visualize the cochlear nucleus below the cerebellum. The cerebellum was sometimes gently retracted to allow a better view. The area of incision was thoroughly infiltrated with Xylocaine after initial preparation. The animal was paralyzed with Flaxedil and respirated through a tracheostomy tube. Pure tone stimuli, 50 msec in duration, were gated on with a 2.5 msec rise-time at a repetition rate of 1/1.4 second and delivered free field in a sound attenuating chamber. Etched tungsten microelectrodes coated with Epoxylite, with impedances of 1-4 megohms, were introduced into the cochlear nucleus by a remotely controlled hydraulic microdrive. Extracellular potentials from 200 μV to 2 mV were recorded.

Thus far 62 single units in DCN and AVCN have been studied. Response latencies varied from 4 to 10 msec from tone onset at the ear for most units. Most tuning curves have been rather broad. The prominent response pattern recorded has been a "primarylike" response, with the neuron responding with increased firing throughout the tone burst. Some of these neurons have also shown a suppression in firing at tone offset. A smaller group of neurons have shown more complex patterns as have been commonly recorded from DCN in unanesthetized preparations by previous investigators: inhibition throughout the tone; no response during the tone with a burst of spikes at tone offset; excitation at certain tone frequencies and inhibition at others; long latency excitatory response continued well after tone offset. Neuron location as judged from depth of electrode penetration from the cochlear nucleus surface and histological location of electrode tracks indicated that a large proportion of the neurons studied have been in DCN. But the predominant response type which has been seen is that usually recorded in AVCN. Ongoing studies, with more precise reconstruction of electrode tracks, are being done to accurately correlate response type with neuron location in cochlear nucleus.

12 CYTOARCHITECTURE OF THE TORUS SEMICIRCULARIS IN THE RED-EARED TURTLE, CHRYSEMYS SCRIPTA ELEGANS. T. FACELLE and R. BROWNER. Dept. of Anatomy, New York Medical College, Valhalla, N.Y. 10595.

The normal cytoarchitecture of the torus semicircularis (TS) was analyzed in eleven turtle brains stained with cresyl violet or impregnated by the Golgi-Kopsch method. Golgi sections of 120μm thickness were studied in the three standard planes. The TS is a midbrain nucleus which caudally bulges on the dorsal mesencephalon and extends rostrally, laterally, and dorsally where it is ventral to the tectal ventricle (TV) at a mid-tectal level. The TV consists of two nuclei: a larger central nucleus (CN) and a laminar nucleus (LN) interposed between the CN and the TV.

The CN has a small neuron population of ovoid-spherical soma ranging from 6.5-14.0μm with one to five primary dendrites. The dendrites radiate from the cell soma without a consistent pattern. This neuronal population has occasional secondary and tertiary dendritic branches with spines. There is a large cell population (16.0-26.0μm) of either ovoid (one-five primary dendrites), or fusiform (two-four dendrites). The large ovoid cells often show secondary and tertiary dendrites with spines seen frequently on the distal dendrites, and infrequently on the primary dendrites and soma. The primary dendrites form different patterns: radiate, bipolar, or perpendicular where two dendrites emerge from the soma at a 90^{o} angle to each other. The fusiform cells have dendrites emerging at opposite poles of the soma and show distal branching, but no dendritic spines. The only notable distribution is the encapsulation of the CN borders by the dendrites of the large ovoid cells at the CN periphery.

At the caudal TS the LN cups the ventromedial CN, more rostrally the LN is medial and then dorsomedial to the CN, and at the rostral pole caps the CN dorsoventrally. In Golgi sections there are three cell types in the LN: ovoid (16.0-19.5μm) with one-three primary dendrites; fusiform neurons (23.0-26.0μm) with two or three primary dendrites; and stellate (20.0μm) with four primary dendrites. All LN cells lack spines but many display an extensive, branching dendritic field with dendrites up to 550μm long. The dendrites of the LN neurons form a criss-crossing grid in three directions: dorsomedial to ventrolateral (from the TV into the CN), dorsolateral to ventromedial (along the dorsal CN border), and anterior to the CN the LN fibers run rostral to caudal.

13 THALAMIC PROJECTIONS TO THE POSTERIOR AUDITORY CORTICAL FIELD IN CAT. K. A. FitzPatrick, T. J. Imig* and R. A. Reale. Dept. of Neurophysiology, Waisman Center, Univ. of Wisconsin, Madison, WI 53706.

Electrophysiological mapping experiments currently in progress in this laboratory have deomonstrated the presence of a secondary auditory field buried within the posterior ectosylvian sulcus in the cat (Reale and Imig, this meeting). This field, called the posterior auditory field, occupies the posterior bank of the sulcus and contains a complete representation of the cochlea. In the present study injections of horseradish peroxidase were made into selected regions of the posterior field after constructing a detailed tonotopic map of this region. The location of labeled cells in the medial geniculate body was analyzed in order to determine the sources of thalamic input to the posterior field. Following injections confined to this field, labeled cells are found primarily in restricted portions of the principal and dorsal nuclei of the medial geniculate. Additional labeled cells appear in the ventrolateral nucleus of the geniculate and an occasional cell is seen in the magnocellular nucleus and nucleus of the brachium of the inferior colliculus.

Supported by NIH Program Project Grant NS12732, Core Grant HD-03352, and NSF grant BNS 76-19893, Postdoctoral Fellowship NS-05232-01.

14 EFFECTS OF HAIR CELL LOSS IN THE WALTZING GUINEA PIG ON THE SYNAPSES OF THE SPIRAL GANGLION CELLS IN THE ROSTRAL AVCN. R.L. Gulley and R.J. Wenthold. Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio and LNO, NIH, Bethesda, MD.

In the NIH strain waltzing guinea pig, there is a progressive loss of hair cells in the organ of Corti beginning 10 days after birth. By 60 days, all hair cells have degenerated. Between days 30 and 90, 60 to 80% of the spiral ganglion cells degenerate. After 90 days, a stable population of both type I and II spiral ganglion cells remains. There is no further loss of ganglion cells during the next 6 months. The terminals of the ganglion cells in the rostral AVCN, the end bulbs of Held, are normal through 20 days of age. In animals older than 90 days, the synaptic junctions are flattened but the terminals otherwise are normal. In these older animals, all stages of granulofilamentous degeneration can be identified in these terminals following surgical destruction of the ganglion cells that remain after hair cell loss. In freeze-fracture replicas, the postsynaptic membranes of the end bulbs before hair cell loss are identical to those in normal guinea pigs. However, the postsynaptic membrane opposite end bulbs in animals older than 90 days have a greater density of nonaggregate particles on the external leaflet than this membrane in animals before hair cell loss. Moreover, in older animals there are no perisynaptic aggregates on the external leaflet of the membrane. The number and distribution of junctional aggregates is identical to that in animals before hair cell loss. The similarity of the changes in the principal cell membrane following hair cell loss to those seen immediately following deafferentation of the principal cell suggests that synaptic activity may be important for maintaining the postsynaptic membrane. Moreover, there is a clear implication that the presence of intact spiral ganglion cells following hair cell loss does not necessarily imply the existence of functionally competent synapses in the CNS.

15 ALLOXAN - INDUCED DIABETES CONFERS PROTECTION AGAINST KANAMYCIN OTOTOXICITY. Paul S. Guth*, Juan Garcia-Quiroga and Charles H. Norris*. Dept. Pharm., Sch. Med., Tulane Univ., New Orleans, LA 70112.

The clinical utility of the aminoglycoside antibiotics is hampered by their well-known capacity to cause labyrinthine damage. The pattern and time course of the damage produced have been extensively described by many researchers, but the ototoxic mechanism remains unclarified. Our attention was focused on the aminosugar moieties of these antibiotics by the finding of Owada (Chemotherapia 5, 227, 1962) that 3-aminoglucose, the aminosugar of kanamycin is itself ototoxic. It is known that aminosugars compete with glucose for transport systems across membranes. As a first test of the hypothesis that the aminoglycoside antibiotics produce their ototoxicity by reducing glucose transport in the organ of Corti we attempted to prevent kanamycin-induced ototoxicity by making animals hyperglycemic. One group of rats were made diabetic with alloxan and were injected with kanamycin (400 mg/kg i.p.) daily. A second group received kanamycin alone at the same dose. Blood and urine glucose levels were monitored periodically. The status of the cochlea was determined once weekly by electrocochleographically measuring the visual detection at 1,2,4,6,8,10,12,14,16, and 32 KHZ. When the non-diabetic animals showed a threshold shift greater than 30db at two frequencies both groups of animals were sacrificed and their cochleas examined by the surface preparation technique. The diabetic group of animals were clearly and dramatically protected against kanamycin ototoxicity as evidenced both by electrocochleography and histological examination. The extent of the protection correlates very clearly with the blood glucose concentration. (Supported by grant 71-6 from the V.A.)

16 EFFECT OF AUDITORY CORTEX ABLATION ON THE PERCEPTION OF MEANINGFUL SOUNDS. Henry E. Heffner. Bureau of Child Research, University of Kansas, & Parsons State Hospital and Training Center, Parsons, KS 67357

Dogs were tested on their ability to classify meaningful sounds before and after auditory cortex ablation. The animals were trained to classify sounds chosen from two well-defined categories of sound. The first category was that of "dog" sounds and the sounds used were dog vocalizations, such as barks, whines, and howls, which had been recorded from over 50 different dogs. The second category was that of "non-dog" sounds and the sounds used consisted of the vocalizations of animals ranging from crickets to elephants as well as the sounds of objects ranging from clocks to steamboats. The sounds were carefully chosen to reduce the possibility that the dogs could classify them simply on the basis of physical characteristics such as frequency spectrum and temporal pattern.

Using a two-choice procedure, the animals were trained to make one response when a dog sound was presented and a different response when a non-dog sound was presented, a task which normal dogs readily learned. The ability of the animals to classify the sounds was tested in two ways. First, the dogs were presented with new sounds and their ability to transfer their training to these sounds was determined. Second, the animals were given an equivalence test in which their response to additional new sounds was determined in the absence of any feedback concerning the correct response. Again, normal dogs were easily able to learn these tasks and could successfully classify as many as 100 new sounds in a single one-hour session.

Bilateral ablation of primary auditory cortex (AI) had no significant effect on the ability of the dogs to classify the sounds. However, bilateral ablation of both primary and secondary auditory cortex resulted in a temporary inability of the animals to discriminate between the sounds as well as to classify them. This disability diminished with time and after several months the animals were able to discriminate and classify the sounds at normal or near-normal levels.

It appears, then, that auditory cortex is not necessary for the discrimination of meaningful sounds. Furthermore, the evidence suggests that bilateral ablation of auditory cortex may result in no more than a transient disruption of the ability to perceive meaningful sounds. (Supported by NIH Grants NS 12 992 and HD 02528, Bureau of Child Research, University of Kansas.)

17 NEUROLOGICAL EVALUATION OF THE AUDITORY SYSTEM IN NONVERBAL SEVERELY RETARDED CHILDREN. Rickye S. Heffner. Bureau of Child Research, University of Kansas, Lawrence, KS 66045.

Recently, we have considered the possibility that the language disorders of nonverbal mentally retarded children may be due in some cases to an inability of auditory input to reach the cortical speech areas of the brain despite normal pure tone sensitivity. To investigate this possibility, nonverbal severely retarded children with normal hearing were tested for their ability to localize sounds. The reason for choosing a sound-localization test was that ablation-behavior experiments have indicated that animals are unable to localize brief sounds if the input from the ear is completely severed from the cortex at any level in the auditory pathway. Therefore, a sound-localization test would constitute a test of the integrity of the auditory pathway from the ear up to and including auditory cortex.

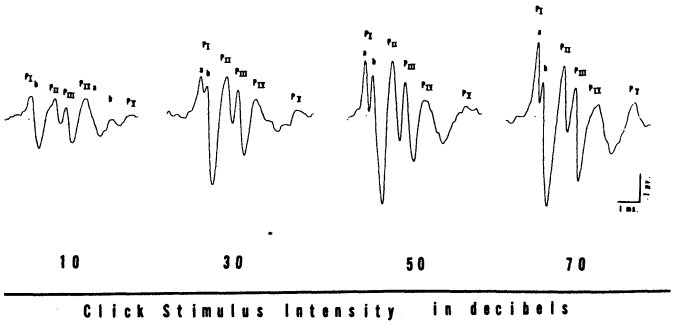
Fifteen nonverbal severely retarded children were tested on their ability to localize single clicks emitted by loudspeakers located to the left and right of midline. The children were trained to respond by touching a screen covering the loudspeaker from which the sound had come. Despite severe cognitive and motor impairments, twelve of the children were able to localize single clicks with normal or near-normal thresholds while only three were unable to localize single clicks at all.

Since disruption of auditory input to the cortex is known to disrupt the localization of brief sounds, it appears that the auditory system of these twelve children is at least sufficiently intact to enable them to localize sounds. Therefore, the inability of these children to learn language cannot be due to gross disconnection of the ears from the cortex. Further work is presently being conducted to determine if the inability of the other three children to localize single clicks is due to an auditory incapacity or to some cognitive, emotional, or motor problem.

(This work was supported by Grants CDRC 1-P01-NS-11601 and HD 02528, Bureau of Child Research, University of Kansas.)

18 DIFFERENTIAL SENSITIVITY OF PERIPHERAL AUDITORY VOLUME CONDUCTED RESPONSES OF THE C57BL/6 MOUSE TO ACOUSTIC STRESS. Kenneth R. Henry. Dept. Psychol., U. of Calif., Davis, 95616.

Volume-conducted inner ear and auditory brainstem responses to a click centered at 2 kHz were obtained from the C57BL/6 mouse. By using a vertex-to-mouth electrode configuration, and maintaining a constant ear temperature (37.5 ± .1°C), it was possible to simultaneously obtain recordings representing cochlear microphonics (CMs) and the negative summating potential, in addition to the auditory nerve and brainstem (P_I to P_V) far field measurements previously described by Jewett. Rarefaction clicks produced a larger CM amplitude and a shorter CN8 (P_I) latency than did condensation clicks. Dual P_I peaks were observed, separated by an interval which approximated the period of the click center frequency. Although P_{Ia} and P_{Ib} showed parallel latency decreases as the click was increased from 30 to 70 db, P_{Ia} amplitude increased while P_{Ib} amplitude decreased as stimulus intensity increased over this range. After exposure to 5 min of 110 db asynchronous noise centered at 5 kHz, all the measures described above showed a decrease of amplitude, and condensation and rarefaction clicks no longer produced differential effects on CMs and P_I measures. One week postexposure, P_{Ib} continued to show an amplitude decrement from 10 to 70 db, while P_{Ia} was only decreased in amplitude at 70 db. Hypothermia and barbituates interacted with noise in these postexposure changes. The hair cell loss due to this acoustic stimulus was greatest in the apical region of the organ of Corti, and it was postulated that P_{Ib} reflects activity which is generated at or near the apical portion, while P_{Ia} represents synchronous activity generated nearer the base of the auditory transducer.



19 A PRELIMINARY STUDY ON THE EFFECTS OF THE HYPEROXIA AND HYPOCAPNIA TO THE 8TH NERVE ACTION POTENTIAL IN CHINCHILLA. S.-M. Hou and D. M. Lipscomb*. Noise Research Laboratory, Dept. of Audiology & Speech Pathology, The University of Tennessee, Knoxville, TN. 37916.

Hyperoxic breathing at 1 atm was found to produce two types of neural depressions in the action potential of the cochlear nerve. A rapid depression of the AP immediately upon flushing the chamber with 100% oxygen was observed. Complete recovery of the AP takes place within 10 seconds of introducing trace amount of carbon dioxide into the inhalant. The second type of neural condition developed over 2 hours of pure oxygen breathing and consists of a prolonged AP depression and latency shift. Recovery from this effect was achieved 6 hours after returning to air. The short term effect was found due to carbon dioxide deprivation during the 100% oxygen inhalation and the long term effect was found to be result from the development of intracellular oxygen toxicity by prolonged hyperoxic breathing. The possible role of the superoxide radical in the auditory oxygen toxicity will be discussed.

20 THE ABILITY OF SINGLE CELLS AND EVOKED POTENTIALS FROM THE CAT'S BRAIN STEM TO RESPOND TO REPETITIVE ACOUSTIC STIMULATION. Chi-Ming Huang* and Jennifer S. Buchwald, (SPON: Lowell E. White, Jr.) Dept. Physiology, Univ. of S. Alabama, College of Medicine, Mobile, AL 36688 and Dept. Physiology, BRI, Mental Retardation Research Ctr., UCLA School of Medicine, Los Angeles, CA 90024.

The classical brain stem auditory relay pathway contains a chain of relay neurons. They appear to make monosynaptic contact with relay neurons in the structure to which they project, thereby establishing a fast conduction pathway with minimum synaptic delay. Their sequential activation provides a basis for the current interpretation of brain stem evoked response (BER). The short response latencies suggest that the synaptic conduction is secure and the axons are probably the fastest-conducting axons in the auditory pathway. Since, in a given brain stem structure the initial discharges of these relay neurons are usually highly synchronized, it also suggests that these neurons may have similar synaptical as well as morphological features such as diameters of axon, types of synapse, etc. This report deals with single neurons in the brain stem of anesthetized adult cats and evoked potentials from the brain stem of anesthetized cats and awake cats. Short (45 ms) tones at the neuron's best frequency were delivered at rates from 1/sec to 20/sec. In a given brain stem structure, depending upon the level, approximately 20 to 50% of all the neurons sampled were short-latency cells. (e.g. 50% in the cochlear nucleus and 20% in the inferior colliculus). The response plasticity of neuronal discharges was analyzed and compared in different neurons. The response plasticity of BER was also analyzed. In the same neuron, the response was broken down in segments of time and analyzed. The data suggested that (1) the initial discharge (within 5 ms of tone stimulus) of short-latency cells demonstrated identical response plasticities as that of the BER. The later discharge of short and long-latency cells demonstrated the same response plasticity. (2) In awake cats, the response plasticity of BER components 1-4 were similar to that measured when the cat was anesthetized. (3) Evoked potentials from the inferior colliculus showed a peculiar enhancing effect in the awake state when the repetitive acoustic stimuli were separated by approximately 500 msec.

These data suggested that the BER may also be under central control.

21 THE ORIGINS AND TARGETS OF CORTICOCORTICAL CONNECTIONS RELATED TO TONOTOPIC MAPS OF CAT AUDITORY CORTEX. T. J. Imig* and R. A. Reale (SPON: A. L. Berman). Dept. of Neurophysiol. and Waisman Ctr. on Mental Retardation, Univ. of Wisconsin, Madison, WI 53706

Within the auditory region of the cat's cerebral cortex are located several complete and orderly frequency representations. In the experiments reported here, we have employed microelectrode mapping techniques to study the frequency organization of the primary auditory field (AI) and two adjacent auditory fields. Adjoining AI caudoventrally is a posterior field (P) which occupies the caudal bank and part of the rostral bank of the posterior ectosylvian sulcus. Bordering AI rostrally is an anterior field (A) located on the anterior ectosylvian gyrus and extending onto the caudal bank of the anterior limb of the suprasylvian sulcus. Within each field is a complete and orderly frequency representation with a narrow band of frequencies represented along a cortical strip. During experiments in which the frequency organizations of these fields were mapped, tritiated (3H) proline was injected at one or more locations into a restricted portion of the frequency representation of AI, A or P. From histological sections processed for autoradiography, maps of the frequency organization of auditory cortex and the distribution of labeled protein were reconstructed. Following an injection of 3H proline into AI, labeled protein is found in both A and P. Following an injection of 3H proline in either A or P, labeled protein is found in AI. Furthermore, frequencies represented at the injection site correspond closely to frequencies represented at target sites. These data indicate that similar portions of the frequency representations in fields AI and P and fields AI and A are reciprocally connected. When projected upon the cortical surface, the heavily labeled region produced by a single injection of 3H proline in field AI, A or P occupies a circular region centered at the injection site. On the other hand, the distribution of labeled protein found at the target sites, when projected upon the cortical surface, occupies an area elongated in a direction parallel to isofrequency contours. Supported by NSF grant 76-19893 and NIH grants NS-12732, HD-03352, NS-05459 and NS-05326.

22 AUTORADIOGRAPHIC DEMONSTRATION OF PRIMARY PROJECTIONS TO CAT COCHLEAR NUCLEUS. Eileen S. Kane and John W. Conlee.* Dept.Anat. University of Chicago, Chicago, Illinois 60637

Manual injections of small volumes (0.15-0.7 µl) of tritiated leucine (25-70 µC) directly into the fenestra rotunda of adult cats, followed by 24-48 hour survivals, perfusion-fixation, frozen-sectioning, and routine processing of mounted sections for light microscopic autoradiography showed clear grain distribution in the ipsilateral cochlear nucleus only. Significant grain counts (above background) occurred in all major regions of ventral cochlear nucleus or VCN (500-600 grains/100 μm^2) with fewer grains (150-300 grains/100 μm^2) in dorsal cochlear nucleus or DCN. Liquid scintillation counts (LSC) of cochlear nerve roots showed significantly greater uptake by experimental vs. control roots (average dpm's = 30:1 after 48 hours) in all cases. Similar results were obtained with and without blockage of the cochlear aqueduct. Specifically, our autoradiographic studies showed more dense and uniform labelling of all major regions of the ventral than the dorsal cochlear nucleus, predominantly perisomatic distribution of label in the anteroventral (AVCN) and posteroventral (PVCN) cochlear nuclei and in the fusiform cell layer (FCL) of DCN plus a dense-to-sparse gradation of grain density from deep to superficial layers of DCN. Heaviest labelling occurred in the nerve root zone and lightest in the superficial DCN. Both bright and, particularly, dark-field illumination, revealed clear grains in emulsion over somata of PVCN (both PA and PP) and in AVCN (both AA and AP), over "giant" and small cell somas of deep DCN, and several grains consistently over fusiform cell somata. Octopus cells were notably covered with nearly uniform grain populations; frequently, linear grains (suggesting incoming axons) could be followed directly into these perisomatic grain clusters. Our observations have confirmed findings of numerous prior investigations of cochlear inputs and have provided the basis for further study of (1) primary synaptic terminals in specific cochlear nucleus regions and (2) primary neurotransmitter substances, now under study. (Supported by University Chicago, Deafness Res. Fdn. and USPHS Grants NS-12071 & NS-00008 (RCDA) to E. S. K.).

23 DISCRIMINATION OF LEFT-RIGHT VS RIGHT-LEFT CLICK PAIRS FOLLOWING LESIONS OF AUDITORY CORTEX IN THE RAT. Jack B. Kelly Department of Psychology, Carleton University, Ottawa, Canada, K1S 5B6.

Lesions of auditory cortex in cats result in severe deficits in the ability to localize sounds in space. Even unilateral lesions produce impairments in localization if the animals are tested with left and right sounds presented in pairs separated by small time intervals, that is,the precedence effect (Cranford, Ravizza, Diamond and Whitfield, 1971). Because the precedence effect is a very sensitive test in the cat, we have employed discriminations of small time intervals between spatially separated loudspeakers to assess the effects of auditory cortical lesions in the rat. In our tests the animals were trained by the conditioned suppression procedure, first, to discriminate a train of left clicks from a train of right clicks emanating from loudspeakers separated by 180°. When the animals had learned this simple left vs right discrimination, they were given further tests with paired clicks in which they had to discriminate left-right from right-left sequences. The temporal separations (Δt) between left and right clicks ranged from 8 msec to 250 microseconds. In spite of the apparent complexity of this stimulus situation, rats with bilateral lesions of auditory cortex could still perform the discrimination with both large and small Δt values. Unilateral lesions also produced no obvious impairment. We have concluded that,for rats,auditory cortex is not essential for discrimination based on the precedence effect. The apparent difference between rats and cats is possibly based upon the nature of the response required by the testing procedure.

24 AUDITORY RECEPTIVE FIELDS IN THE OWL. Eric I. Knudsen and Masakazu Konishi. Div. Biology, California Institute of Technology, Pasadena, CA 91125.

The influence of sound location on the responses of auditory neurons in the barn owl (Tyto alba) were studied using noise, clicks, tones and FM stimuli delivered by a movable speaker under free-field, anechoic conditions. Three types of units have been found: 1. space-independent, 2. space-preferring, and 3. space-dependent. Space-independent units were not selective for sound location. Space-preferring units responded strongly to sounds from a particular area of space and sporadically to sounds from outside this area, but their responsive areas expanded with increasing sound intensity. Space-dependent units demonstrated well-defined receptive fields that were relatively independent of sound intensity, expanding by less than 20° in azimuth and 45° in elevation to a 20 dB increase in sound intensity. The typical receptive field was vertically elongate, although some circular and irregular fields were found. The smallest receptive fields subtended as little as 8° in azimuth and 20° in elevation. Just as with space-preferring units, the vigor of space-dependent unit responses increased as the sound stimulus approached the center of their receptive fields.

All three unit types were found in the telencephalon (space-preferring > space-independent > space-dependent). In contrast, only space-dependent units with small receptive fields were found in a small region of the midbrain. These units all shared similar characteristic frequencies (5.5-8.5 KHz) and responses to noise and click stimuli. The single attribute found, in which they differed, was the location of their receptive fields which moved systematically as a function of electrode position. These response properties argue strongly for this midbrain region being involved in sound localization. Furthermore, the distribution of receptive fields in this midbrain region was heavily biased toward a limited area directly in front of the owl, suggesting that within this frontal area of "expanded representation" the owl attains maximal spatial acuity.

25 RESPONSES OF INFERIOR COLLICULUS NEURONS TO INTERAURAL TIME DIFFERENCES IN THE CAT. S. Kuwada*, T.J. Buunen*, J. Syka* and R. Wickesberg* (Spon. H. Sobkowicz) Dept. of Neurophysiology, Univ. of Wisconsin Med. School, Madison, Wisconsin 53706.

Several studies have reported the existence of inferior collicular neurons whose responses vary cyclically as a function of interaural time delay. The interval between peaks of this cyclic function equals the period of the stimulating frequency. Many of these cells repond maximally or minimally to a specific time delay independent of the stimulating frequency or intensity. Neurons which have the above properties are said to exhibit "characteristic delay". The present experiment describes a population of inferior collicular neurons which display cyclical properties but for which the interaural time delay resulting in maximal firing changes as a function of frequency and intensity. A computer system was employed for stimulus generation and data collection. Our protocol was as follows: 1) Isolation of a unit that responds cyclically to interaural time delays. 2) Binaural presentation of pure tones of the same frequency and intensity while varying the interaural time delay. Both the ipsilateral and contralateral stimuli were systematically delayed covering two periods of the stimulating frequency in steps of no more than .1 of a cycle. This procedure was continued until the frequency range of a particular cell was adequately sampled for a given SPL level. For any particular presentation the stimuli to both ears were identical in frequency and intensity. 3) Time permitting, we set several intensity differences between stimuli and repeated the time delay sequences. 4) Finally, monaural and binaural response areas were determined. All the units studied displayed a frequency related delay: more specifically, the maximal response of a neuron occurs at delays which were an orderly function of frequency. Within this population, there was a small number of cells which responded optimally to a particular phase difference between the binaural stimuli across frequencies. We termed such a response "characteristic phase". The interaural delay resulting in maximal firing could be shifted as much as 1/5 of a period as a function of intensity difference between the binaural stimuli.

We conclude that: 1) The delay eliciting maximal firing can be frequency dependent; 2) For some cells, the maximal response occurs at a particular phase difference independent of frequency; 3) Interaural intensity differences at a given frequency can shift the delay at which maximal response occurs.

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26 CLICK-EVOKED NEAR- AND FAR-FIELD POTENTIALS AND MULTIPLE UNIT ACTIVITY RECORDED FROM MONKEY SUPERIOR TEMPORAL PLANE AND SUBCORTICAL STRUCTURES. Alan D. Legatt, Joseph Arezzo*, and Herbert G. Vaughan, Jr.* Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Short latency far-field click-evoked potentials have been recorded in several species, including man. Based in part on depth studies in cat and rat, these potentials are believed to reflect neuronal activity in subcortical structures which precedes cortical activation. We examined this issue in the primate by intracranial mapping of gross potentials in monkeys, and by comparison of the far-field activity to the near fields and multiple unit activity generated in the primary auditory cortex.

Following 100 μsec 95 dBSPL peak clicks delivered at 10 per second monaurally to the anesthetized monkey, a series of seven positive deflections are seen at the vertex (reference = mastoid ipsilateral to the stimulated ear) which resemble the human far-field potentials, although with a shorter time course. The peak latencies are 1.6, 2.6, 3.4, 3.8, 4.4, 6.1, and 7.8 msec. Other electrode placements give complex waveforms which may have additional peaks; topographic analysis of these data provides information about the locations and orientations of the generators.

Of the seven waves recorded at the vertex, the first five precede activity in the superior temporal plane, and can be recorded in the vicinity of the latter structure as far fields. Topographic analysis confirms that these are generated in subcortical structures; amplitude increases of greater than 300-fold are seen as the recording electrodes are moved from the vertex through the brain stem. Inferences about the sources of the latter two components are more difficult, however, as they overlap in time with fields generated in the superior temporal plane. Brain stem structures show activity patterns which begin with latencies as short as those of the far-field potentials but continue well into the cortical response.

Multiple unit activity recorded in the superior temporal plane remains at the prestimulus baseline level until 5.0 msec after the stimulus, when it begins to increase sharply. It peaks at 5.8 msec at a level 22% above baseline, and then declines, almost reaching the baseline at 6.1 msec. Subsequently the multiple unit activity increases to more than twice the baseline level, followed by a decline to below baseline. The initial increase in multiple unit activity, which partially overlaps the sixth wave of the early evoked potential, may represent geniculocortical activity as well as the initial activation of cortical neurons.

(This work was supported by training grant #5T5-GM-1674 from NIGMS and by grant #MH06723 from NIMH.)

27 AUDITORY DYSFUNCTION FOLLOWING HUMAN HEAD INJURY: EVALUATION OF MONAURAL VERSUS BINAURAL AUDITORY FAR FIELD POTENTIALS. Barbara White Navari*, Richard P. Greenberg and Donald P. Becker. Div. of Neurosurg., Medical College of Virginia, Richmond, VA 23298.

To determine the accuracy with which auditory far field evoked potentials (Jewett, Brain 94:681-696, 1971) could localize auditory system dysfunction in comatose head injury patients we obtained both monaural and binaural auditory far field potentials from 33 patients with severe head trauma and correlated the evoked potential data with the patients clinical auditory status 3 months or more after head injury. (Greenberg, J. Neurosurg. 46, 1977). All patients were unresponsive when studied (mean day 3 post-injury) and the presence or absence of auditory dysfunction was clinically unknown. When the auditory far field data was evaluated it was noted that the evoked potentials generated by monaural stimulation of the ears significantly associated with the presence or absence of auditory dysfunction in the 33 patients ($p<.001$; Fisher's Exact Test) while the auditory far field evoked potentials generated with binaural stimulation did not. Therefore, the monaural and binaural evoked potential data were examined to determine the basis for this disparity.

If the waveforms and latencies of the first VII positive potentials generated after monaural stimulation of the left and right ear were similar the data generated by binaural stimulation correlated with the presence or absence of auditory dysfunction in the patients. However, when latency differences were present after monaural stimulation, comparing the left to the right ear, the binaurally produced data in the same patient did not correlate with auditory dysfunction. Latencies of the binaural far field potentials in these cases were found to have intermediate values between the latencies of each ear stimulated monaurally. For example, stimulation of one patients' left ear produced a wave V latency of 6.7 msec while the wave V latency following right ear stimulation was 5.8 msec. With binaural stimulation wave V latency in this patient was 6.2 msec.

These data suggest that binaural stimulation is unnecessary for adequate evaluation of auditory function following head injury and that it may actually be misleading. (Supported by N.I.H. grant NS 12587 and a Southern Medical Association Training Grant).

28 A COMPARATIVE SCANNING ELECTRON MICROSCOPIC STUDY OF THE OTOLITHIC ORGANS IN FISHES. Arthur N. Popper. Lab. of Sensory Sciences and Dept. of Zoology, University of Hawaii, Honolulu, Hawaii 96822.

Investigations have been conducted on the hair cell orientation patterns of the otolithic organs in the ears of more than 30 species of fish. Species were selected for study on the basis of diversity in acoustic behavior, habitat, and taxonomic relationship.

Inter-specific variation in hair cell orientation pattern is wide, particularly in the presumed auditory regions, the sacculus and lagena. Considerably less inter-specific variation is found in the utriculus where the patterns are very similar to those found in tetrapods.

In most non-ostariophysines (species not having a direct mechanical coupling between the swim bladder and inner ear) the sacculus is divided into four 'regions' with all of the hair cells in each region oriented in the same direction. Generally the cells on the dorsal side of the posterior half of the saccular macula are oriented with the eccentrically placed kinocilium on the dorsal side of the cell while the cells on the ventral side of the posterior half of the macula are oriented ventrally. Cells on the anterior half of the macula are divided into two groups, one oriented anteriorly and one posteriorly. The relationship between the anteriorly and posteriorly oriented groups varies considerably in different species. There is also considerable inter-specific variation in the relationship between the sensory region and overlying otolith, with the otolith, in many species, not covering the whole sensory area.

The hair cells on the lagena are generally divided into two groups oriented in opposite directions. The relationship between these groups varies in different species, but variation is not as extensive as in the sacculus.

The functional and taxonomic significance of the diversity of hair cell orientation patterns among fishes is not yet clear. However, it is possible that the different patterns relate to inter-specific variation in the method of sound detection and initial processing of acoustic signals. It is also likely that fishes can perform detection of sound source direction (sound localization) and the hair cell patterns may have evolved in relationship to such mechanisms in at least some non-ostariophysines. Thus the different patterns, particularly in the sacculus, may represent different approaches taken in the evolution of various teleosts for making maximum use of sound directional information. (Supported by NINCDS and the Deafness Research Foundation.)

29 AN ORDERLY FREQUENCY REPRESENTATION IN THE POSTERIOR ECTOSYLVIAN SULCUS OF THE CAT. Richard A. Reale and Thomas J. Imig*. Dept. of Neurophysiology, Waisman Center on Mental Retardation and Human Development, Univ. of Wisconsin, Madison, Wis. 53706.

On the basis of electrophysiological and cytoarchitectural evidence the auditory cortex of cat has been divided into a primary field (AI) and several surrounding secondary fields. These auditory fields are not confined to the gyral surfaces but are also located within the three major sulci in this region. We have undertaken a microelectrode investigation of one secondary area which we call the posterior field (P). P lies on the banks of the posterior ectosylvian sulcus (PES) where it is bordered dorsorostrally by AI. This field covers approximately 40 mm^2 of cortical surface, an area nearly equal to that occupied by AI. Electrode penetrations directed into both the anterior and posterior banks of the PES were aligned nearly parallel to these sucal walls. The responses of single neurons or neuron clusters to tone pips were studied at intervals of 300 micron or less. Best frequencies, latencies of responses at several stimulus intensities and occassionally binaural interactions were determined at each location. These data together with the histological reconstruction of each animal's brain revealed a complete and orderly frequency representation in P. Low frequencies in this field are located dorsorostrally contiguous with the low frequency representation in AI. High frequencies in P are located caudoventrally occupying a portion of the posterior bank of the PES and often extending upon the posterior ectosylvian gyrus. Thus the frequency organization of P is a mirror image of the frequency organization in AI. Often isofrequency contours extend from the posterior bank of the PES across the bottom of the sulcus onto the anterior bank. In the anterior and middle portions of P, neuronal responses to near threshold stimulation consist of a short latency (15-30 msec) burst of firing confined to a restricted frequency range. In the posterior portion of P, responses latencies of 50-150 msec are often seen in addition to the short latency component. These long latency discharges occur over a broad range of the frequency spectrum, making a determination of best frequency difficult or impossible. Supported by NIH Program Project Grant NS-12732, Core Grant HD-03352, Postdoctoral Fellowship NS-05459-01 and NSF Grant BNS 76-19893.

30 THE ROLE OF AFFERENT FIBER SYSTEMS ON THE DEVELOPMENT AND DIFFERENTIATION OF THE INFERIOR COLLICULUS. D.K. Ryugo. Dept. Anatomy, Univ. Vermont, Burlington, Vt 05401.

The present study investigates the role played by afferent fiber systems in the formation and differentiation of the nuclear subdivisions within the inferior colliculus(IC) of the rat. It has previously been reported that deafferentation of the central nucleus(CN) of the IC on post-natal day 1(PN 1) produces a delamination of the CN principal neurons and their dendrites, a shrinkage in CN volume and an apparent expansion of the pericentral nucleus(PcN)(Killackey and Ryugo, Anat. Rec., 187:624, 1977). Deafferentation of PcN was performed by auditory cortex ablations on PN 1 in an attempt to detect shrinkage in PcN with a corresponding expansion of CN; however, no effect on either PcN or CN was observed following such lesions. Golgi examination of CN on PN 1 showed the presence of lateral lemniscal fibers and a laminar arrangement of the immature principal neurons and their dendrites; electron microscopy at this age revealed only the start of synaptogenesis within the neuropil. In the adult, Fink-Heimer analysis following spinal cord hemisections, dorsal column nucleus lesions and auditory cortex ablations revealed terminal degeneration converging in PcN and the external nucleus(XN). A small amount of degeneration was also observed in CN. Sections of the IC commissure resulted in heavy degeneration in the dorsal medial nucleus(DM), PcN, XN and the dorsal portion of CN; the ventral portion of CN was entirely free of commissural projections. Sections of the lateral lemniscus produced heavy degeneration in CN, moderate degeneration in DM, PcN and XN, and sparse degeneration in the contralateral DM, PcN and XN.

These observations prompted the following conclusions: 1) the principal neurons and their dendrites of CN are oriented in a laminar pattern as early as PN 1; 2) CN lamina initiation does not appear to be dependent upon synaptic contacts; 3) the maintenance of CN lamination does depend upon the presence of lateral lemniscal fibers, at least for the age group studied; 4) since the entire CN is laminated, the differential distribution of commissural afferents within CN indicates that they probably do not play a crucial role in the formation or maintenance of the laminae; 5) the lack of shrinkage in PcN following auditory cortex ablations may be attributable to the sustaining influence of the remaining fiber systems.

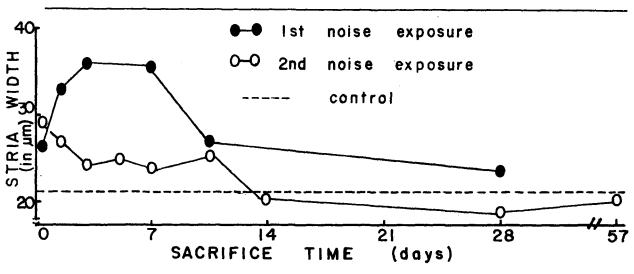
(Supported by UVM Research Development Award)

31 STRIA VASCULARIS PATHOLOGY AND RECOVERY FOLLOWING NOISE EXPOSURE. P.A. SANTI AND A.J. DUVALL*. Dept. Otolaryngology, Sch. Med., U. Minn., Minneapolis, MN 55414.

Duvall et al., 1974 (Ann. Oto-Rhino-Laryng. 83: 498) previously described stria vascularis pathology and recovery following a noise exposure of 700-2800 Hz at 123dB SPL for 15 min. The purpose of this research was to obtain quantitative data on the time course of stria pathology and recovery following 1 or 2 equivalent noise exposures. The second noise exposure was given 28 days after the first when stria morphology appeared to have returned to normal.

Sixty-one chinchillas were noise exposed and their right cochleae processed for transmission electron microscopy at sacrifice times of 0,3,6,24 hr, 3,7,10,14,28 days following the noise exposures. Each cochlea was bisected mid-modiolar and a segment of the stria removed from each of the three cochlear turns. Stria damage, consisting primarily of intercellular edema, was measured, using a light microscope eyepiece micrometer, from 2 µm epon sections. The results of these data are shown in the figure below. Although both noise exposures were equivalent and stria morphology appeared normal before the second noise exposure was given, stria edema was significantly less after the second noise exposure. Twenty eight days after the second noise exposure stria thickness had still not returned to normal. An additional group of animals were sacrificed 57 days post second exposure and showed that stria thickness was slowly returning to normal.

Six chinchillas have also been injected i.v. with horseradish peroxidase (Sigma type II, 0.1 mg/g body weight) to demonstrate stria blood vessels. These animals were sacrificed three days following a single noise exposure. Surface preparations revealed that stria damage was 1) diffusely distributed, 2) began in the lower basal turn near the supra strial region, and 3) progressively increased towards the cochlear apex. Presently, thin sections of selected tissues are being examined electron microscopically.



32 NON-PRIMARY AFFERENTS IN THE ROSTRAL AVCN OF THE GUINEA PIG. A.M. Schwartz* and R.L. Gulley (SPON: K.E. Alley). Dept. of Anatomy, Case Western Reserve University, Cleveland, Ohio 44106.

About 50% of the soma of the principal cell of the rostral anteroventral cochlear nucleus (AVCN) in the adult guinea pig is covered by large, primary terminals, the end bulbs of Held. In addition to the end bulb, 3 other axosomatic boutons have been identified in thin sections and freeze-fracture replicas. These boutons are non-primary, since all 3 types have been identified in animals 30 days following complete unilateral cochlear ablation, when all end bulbs have degenerated. Four percent of the principal cell soma is covered by small, angular boutons which often occur singly. Typically, these boutons are not surrounded by glial processes. These boutons contain small, round synaptic vesicles. The long, flat synaptic junction is undistinguished except for symmetric densities lining the cytoplasmic surface of the membranes. These boutons are more commonly found forming axo-dendritic synapses in the neuropil. About 12% of the soma is covered by small to medium-sized boutons containing oval synaptic vesicles. Single boutons of this type are isolated on the soma by astrocytic processes. In freeze-fracture replicas, each of these boutons has one to three crescent-shaped synaptic junctions. Accordingly, the size and shape of the junctions in thin sections is variable. The cytoplasmic density lining the postsynaptic membrane at the synaptic junctions is slightly thicker than the corresponding region of the presynaptic membrane. About 6% of the soma is covered by small boutons containing distinctly flattened synaptic vesicles. These boutons frequently occur in clusters which are ensheathed by astrocytic processes; however the individual boutons within the cluster are not separated by glial processes. These boutons have a flattened, oval-shaped synaptic junction. In thin sections, the cytoplasmic surface of the membranes at the synaptic junction are lined symmetrically by dense material. In freeze-fracture replicas, there is no specialization of the post-synaptic membrane opposite any of the non-primary boutons. Boutons containing flattened vesicles, in animals kept in a sound deprived environment for one hour prior to sacrifice, have a greater number of synaptic vesicle attachment sites, indicative of synaptic vesicle release, than these boutons in animals in a noise enriched environment. This observation suggests that this population of terminals on the principal cells may be active in the absence of sound. Since the other non-primary terminals can be easily distinguished using the criteria described above, it is hoped that further studies using the freeze-fracture technique under well defined physiological conditions, will elucidate the role of the other inputs to the principal cell of the rostral AVCN.

33 OTOTOXICITY AS MEASURED BY THE AUDITORY BRAINSTEM RESPONSE AND THE SCALP RECORDED COCHLEAR MICROPHONIC IN GUINEA PIGS. Vincent L. Schwent*, John S. Williston, and Don L. Jewett (SPON: A.S. Gevins). Dept. Ortho. Surg., Sch. Med., UCSF, San Francisco, CA. 94143.

The ability of the ABR (Auditory Brainstem Response) and the SRCM (Scalp Recorded Cochlear Microphonic) to reflect the ototoxic effects of kanamycin sulfate (s.c.) was assessed using adult guinea pigs. Twenty-one albino guinea pigs were studied with one third receiving 400 mg/kg/day for 7 consecutive days, one third receiving 200 mg/kg/day for 14 days and the remaining third receiving no drug. ABRs were recorded from vertex to stimulated ear by standard response-averaging methods using 50μsec clicks at 40 and 80 dBSPL at both 10 and 80 Hz repetition rates. Responses were obtained before, during and after drug administration with recording from most animals continuing for at least 2 months after the kanamycin treatment. At the termination of the study additional intensities were also recorded. SRCMs were continuous pure tones were recorded from the vertex referenced to the neck or throat by a special modification of our averaging techniques (see Neuroscience Ab. 2:25, 1976). SRCMs were obtained to 500 Hz (90 dBSPL), 2 KHz (90 dBSPL) and 8 KHz (90 and 100 dBSPL) with continuous tones. Hair cell counts of representative cochleas are pending.

Several guinea pigs in both the high dose (400 mg/kg) and low dose (200 mg/kg) groups evidenced varying degrees of change in the ABRs after kanamycin administration, usually by increased component latencies. The time course of these latency increases and their interaction with stimulus intensity produced widely varying patterns of auditory system change among those animals evidencing ototoxicity. The relative amplitude of the SRCM to the three frequencies of tonal stimulation showed differential frequency effects in some animals. The implications of employing the ABR and SRCM to monitor ototoxicity in non-communicative patients receiving potentially ototoxic drug (e.g. premature infants) and in animal studies on the effects of such drugs will be discussed.

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34 DETECTION OF THE PRIMARY AFFERENT AUDITORY TRANSMITTER USING BIOLOGICAL ASSAY. William F. Sewell and Paul S. Guth*. Dept. Pharmacology, School of Medicine, Tulane University, New Orleans, La. 70112

The junction between the auditory hair cells and the primary afferent nerve has the morphological and electrophysiological characteristics of a chemically transmitting synapse. Although several candidates, including gamma-aminobutyrate and acetylcholine, have been put forward as the primary transmitter (PAT) of audition, none has been supported by the evidence. On the contrary, there is evidence that acetylcholine is not the PAT. Unsuccessful attempts at blocking transmission at this synapse with standard pharmacological antagonists have led to the conclusion that the PAT may not be one of the standard transmitters. Consequently, a research strategy which could detect the presence of the transmitter, even though its identity was unknown, was required in order to allow purification and characterization of the transmitter. Frogs were immobilized with d-tubocurarine and the posterior branch of the VIII nerve and the perilymphatic sac surgically exposed. Single unit activity in VIII nerve fibers from the basilar or amphibian papillae was monitored and the units identified by their characteristic frequencies. The frogs were stimulated with white noise or kept in silence while the perilymphatic space was perfused by a push-pull cannula containing artificial perilymph. The collected fluid was then reinfused in silence and the firing pattern of the single unit fiber was monitored and recorded. In several instances, the reinfusion in silence of fluid collected during white noise stimulation resulted in an increase in the firing rate of the single unit being investigated. By this means, the PAT can be collected and attempts at concentration and purification can be monitored by following the effect upon the firing rate of VIII nerve single units. (This work was supported by Grant 71-6 from the Veterans Administration).

35 MAPPING OF THE TONOTOPIC ORGANIZATION OF THE AUDITORY SYSTEM BY UPTAKE OF RADIOACTIVE METABOLITES. M.S. Silverman*, A.E. Hendrickson, B.M. Clopton* Dep't of Otolaryngology and Opthalmology, Univ. of Washington, Seattle, Washington 98195.

Recent studies have shown that neuronal activity within the central nervous system is closely associated with the uptake of deoxyglucose (DG) (Kennedy et al, PNAS, 1976). We have used this association in conjunction with autoradiographic techniques to study tonotopic organization within the auditory system. Pentobarbital-anesthetized rats or guinea pigs were exposed to a pulsed tone for 45 minutes following an intravenous injection of C^{14} or H^3-DG. Immediately following exposure to the tone the brain was removed and frozen in liquid nitrogen-chilled freon and sectioned at 10μ in a cryostat maintained at -20°C. The sections were heat-treated and autoradiographed using X-ray film. Examination of the autoradiographs revealed distinct, localized variations in silver grain density throughout the auditory nuclei. These regional variations of grain density and their relative positioning in a given auditory nucleus are dependent on the exposure tone frequency used following the DG injection. In addition, the autoradiography patterns agree with classical tonotopic mapping as determined by electrophysiological techniques. The use of radioactively labeled DG in conjunction with autoradiography can produce a pictorial and spatial representation of the tonotopic organization of the auditory system that is a powerful alternative to electrophysical techniques. The localization of C^{14} DG in central auditory nuclei will be compared with autoradiographic labeling of auditory pathways after injection of H^3 amino acids. (Supported by grants EY01208 and NS13052-02).

36 DENDRITIC STRUCTURE IN N. LAMINARIS OF THE CHICKEN. D. J. Smith* and E. W Rubel, Depts. of Otolaryngology and Physiology, Univ. of Virginia Medical School, Charlottesville, VA., 22901.

In the avian brain stem, n. laminaris (NL) is composed of a monolayer sheet of 3rd-order auditory neurons receiving spatially segregated binaural input from corresponding regions of the ipsilateral and contralateral magnocellular nuclei. Previous studies have described the functional and structural organization of inputs to NL as well as its tonotopic organization. The present investigation examines the normal morphology of NL cells as a function of their position along the tonotopic dimension of the nucleus in 5-day-old hatchling chickens. Rapid Golgi, Golgi-Cox and Golgi-Kopsch methods were used, the latter providing the most reliable impregnation with little background staining. In the anteromedial portion of NL, which is responsive to high frequencies (2.5-4 kHz), many (16+) short, stubby, unbranched dendrites extend about 16μ from each side of the cell body, giving it a two-sided "thistle" appearance. In the central (1.5-2.5 kHz) region, similar cells have fewer (10-16) primary dendrites which extend further (33μ) from the cell body and are more branched. Toward the posterior pole (below 1.5kHz), 3-8 longer processes branch profusely after extending 50-80μ from the cell body. Quantitative analyses of dendritic lengths were made of 81 neurons sampled from all parts of NL. Camera lucida tracings made down the center of each individual dendritic process were measured with an electronic planimeter. Dorsal, ventral and total dendritic lengths were related to the position of the cell within the rostrocaudal and mediolateral nuclear dimensions. Regression analyses yielded a highly reliable ($p<.001$) 2.5-fold increase in dendritic lengths from the anteromedial to the posterolateral pole of NL. Mean dorsal and ventral dendritic lengths over nuclear regions were also highly correlated ($r= .62$). These results indicate that there is a systematic variation of dendritic morphology coincident with the tonotopic organization of NL. In addition, there is surprising uniformity of dorsal and ventral dendritic lengths within each region of NL.

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37 TIME DOMAIN ANALYSIS OF SINGLE UNITS IN THE INFERIOR COLLICULUS. E. E. Sutter, H. L. Hosford* and B. Dawson*. Hearing and Speach Sciences, Stanford Medical Center, Stanford, CA 94305.

The technique developed by Lee and Schetzen (Int. J. Control 2: 237, 1965) for the determination of transfer characteristics was employed in a study of the response of single units in the inferior colliculus. Recordings were obtained from three chronic awake cats binaurally stimulated with uncorrelated Gaussian white noise. So far, only first order processing has been performed on the data. The first order correlation provides information concerning latencies, "memory", and tuning. Since the response is a spike train, the first order correlation is represented by the spike locked average of the noise stimuli. Averages of the input signals to the ipsilateral and contralateral ear over approximately 10,000 spikes were obtained for each unit. For 75% of the cells these averages agreed well with tuning curves obtained by means of pure tone bursts. The absence of strong signal averages for 25% of those cells which responded strongly to tone bursts suggest poor phase-locking of the spikes. In those cases, higher order analysis of the data can be expected to yield valuable information.

38 PERCEPTUAL CONSTANCY FOR VOWEL CONTRASTS IN NORMAL AND LANGUAGE DELAYED CHILDREN. P. Tallal* and R.E. Stark* (SPON: M.S. Preston). Dept. Neurol., Johns Hopkins Med. Sch. Baltimore, MD 21205.

The ability to generalize phonemes across various vocal tracts is an essential aspect of normal speech perception. The child must learn that, although acoustically different, a speech sound produced by different individuals is still the same speech sound. Kuhl (1976, JASA, 60, S90A) has recently demonstrated that infants as young as six months old are able to generalize phonetically similar speech sounds across various size vocal tracts.

Ten children 7½ to 8½ years old with specific developmental language delay and ten normally developing children matched for age, I.Q and socioeconomic status were presented with the vowel /a/ spoken by a man, a woman and a child. Subjects were trained to press the bottom button on a response panel to each presentation of this vowel, regardless of which speaker produced it. Next, subjects were presented with the vowel /ɛ/ spoken by the same man, woman and child and trained to press the top button on the response panel to each presentation of this vowel. Finally subjects were presented with the two vowels produced by the three different speakers in random order and they were required to press the appropriate button on the response panel. No verbal instructions were given at anytime to any of the subjects. Knowledge of results and correction of errors were given and correct responses were rewarded after each trial until a criterion of 20 out of 24 consecutive trials correct had been reached or 48 trials had been given.

Results showed that there was no significant difference between the language delayed and normally developing children's ability to learn to press the appropriate button for each vowel when they were produced by a single speaker. Thus, both groups of children were able to discriminate between the two vowels and learn to associate them with the correct button. However, when the same vowels were produced by various speakers and presented randomly the performance of the two groups was significantly different ($p < 0.05$). Whereas nine out of ten of the normally developing children learned the concept underlying this task and reached the criterion within the 48 trials given, only four out of ten of the language delayed children were able to meet this criterion. The importance of perceptual constancy for normal language development will be discussed.

39 BRAINSTEM PATHWAYS MEDIATING HEAD ORIENTATION TO SOUND. G. C. Thompson and R. B. Masterton. Dept. Psych., Florida State Univ., Tallahassee, Fla. 32306.

The neuroanatomical pathways responsible for the reflexive head-turn toward the source of an unexpected sound have been traced with the ablation-behavior method. Postoperative measurements of latency and accuracy of the orienting response have been made in cats with unilateral lesions of either the lateral lemniscus and pathways medial and rostral to it, the brachium of the inferior colliculus, the midbrain pathways lying medial and ventromedial to the brachium, the superior colliculus, or auditory cortex.

The results show: 1) lesions transecting the lateral lemniscus and nearby pathways (e.g., the dorsal acoustic stria) completely abolish head orientation toward a sound source in the contralateral auditory field; 2) lesions confined to the lateral lemniscus itself disrupt the accuracy of alignment toward a contralateral sound source without affecting the latency or direction of the response; 3) lesions at the level of the brachium of the inferior colliculus including the pathways medial and ventromedial to it abolish the accuracy of the orienting response toward the contralateral field and lengthen the latency of the response; 4) lesions confined to the brachium of the inferior colliculus and the pathways medial to it have little effect on the orienting response except to increase its latency bilaterally; 5) lesions of the superior colliculus including its deepest layers that completely abolish head-orientation to a visual stimulus do not affect head-orientation to a sound stimulus; 6) lesions of auditory cortex including the most ventral auditory fields reduce the probability but not the parameters of orientation to sound in the contralateral field. Comparisons of midbrain lesions that affect the accuracy of orientation with lesions in the same vicinity that do not affect orientation suggest a critical pathway through the midbrain tegmentum ventromedial to the brachium of the inferior colliculus and ventral to even the deepest layers of the superior colliculus. (Supported by NIH:NS-7726)

40 COMBINED GOLGI, HORSERADISH PEROXIDASE (HRP), AND ELECTRON MICROSCOPIC STUDY OF BUSHY CELLS IN THE COCHLEAR NUCLEUS. Leslie P. Tolbert and D. Kent Morest. Depts. Anat., Harvard Medical Sch., Boston, MA 02115, and U. Conn., Farmington, CT 06032.

Golgi impregnations of the posterior part of the cat's anteroventral cochlear nucleus (AVCNp) reveal two types of neurons, bushy cells with short bush-like dendrites and stellate cells with long, tapered processes; Nissl stains reveal globular neurons and multipolar neurons with dispersed and clumped ribosomal patterns, respectively. The present findings indicate that bushy cells are globular neurons that project to the contralateral medial nucleus of the trapezoid body (MNTB). After HRP injections in the MNTB or transected trapezoid body, two types of HRP labelling were observed: diffuse and granular. In the ipsilateral AVCNp, retrograde diffuse labelling of neurons yielded Golgi-like profiles of bushy cells with thick axons projecting toward the trapezoid body. Anterograde diffuse labelling of thick fibers demonstrated typical calyces in the contralateral MNTB. The diffusely filled bushy cells were examined in the electron microscope to establish correlations between ultrastructural observations and Golgi and Nissl findings. Bushy cell bodies were found to have the dispersed ribosomal pattern of globular neurons and to receive several types of synaptic endings, including the large terminals indicative of endbulbs from cochlear nerve axons. In the contralateral AVCNp, cell bodies were labelled with a granular reaction product, presumably due to HRP uptake at synaptic endings; they were Nissl-stained and seen to be globular neurons. It is concluded that bushy cells typically correspond to globular cells, which receive endbulbs from the cochlea and send thick axons to the contralateral MNTB, where they form calyces on principal cells. Principal cells, in turn, project to the nucleus of origin of the crossed olivocochlear bundle, which feeds back to the cochlea. Thus, an anatomical circuit can be defined in terms of specific cell types and kinds of synaptic endings at the ultrastructural level. In this circuit, correlations between particular synaptic patterns and certain physiological signal transfer characteristics are suggested. (Supported by PHS grants NS 06115, NS 13126, GM 00406, and MH 14275.)

41 THE TEMPORAL PATTERN OF DISCHARGES ELICITED FROM LATERAL SUPERIOR OLIVE UNITS WITH ACOUSTIC STIMULATION. Chiyeko Tsuchitani. Sensory Sciences Center, Univ. of Texas Health Sciences Center, Houston, TX, 77025.

The temporal characteristics of the discharges of lateral superior olivary units to acoustic stimulation were examined in barbiturate anesthetized cats. Gold-plated, stainless steel microelectrodes with platinum-blacked tips were used to record extracellularly from units confirmed histologically to be located within the lateral superior olive.

With monaural tone burst stimulation of the ipsilateral ear lateral superior olive units with characteristic frequency, (CF: the frequency to which a unit is most sensitive), greater than 2 kHz produced discharges that were time-locked to stimulus onset (chopper pattern). The initial peaks of the chopper-type poststimulus time (PST) histograms of these units are narrow and are separated by short interpeak intervals. The chopper pattern is not maintained for the duration of a 60 msec tone burst and may be produced by only the first 3 to 13 spikes of the elicited discharges. The discharges following this initial, transient chopper response may drop to very low rates, producing an almost phasic-type PST histogram, or may be maintained at high levels with an irregular temporal pattern. The interspike interval (ISI) histograms of the tone burst elicited discharges are often bimodal in distribution, with a short interval peak formed by the initial chopper response, and a second, longer interval peak formed by the later discharges.

Increasing stimulus level increases the duration of the chopper response and narrows the peak widths and interpeak intervals of the chopper response. With increases in stimulus level the two peaks of the bimodal ISI histograms become indistinguishable and often fuse at high stimulus levels to form a unimodal, symmetrical distribution. Stimulation with tone bursts at frequencies other than unit CF elicite discharges similar to those elicited by CF tones at lower stimulus levels. Simultaneous stimulation of the contralateral ear, which is inhibitory,disrupts the chopper pattern and produces an irregular discharge pattern.

42 AUDITORY NEURONS OF THE MOUSE SELECTIVELY RESPONSIVE TO LONG DURATION STIMULI. James F. Willott and Gregory P. Urban*. Dept. Psychol., Northern Illinois Univ., DeKalb, Ill. 60115.

Obvious means by which the auditory system could encode the duration of acoustic stimuli are the duration of sustained evoked discharges and phasic on-off responses signalling the beginning and end of a stimulus. Both involve changes in neural activity closely following the onset and termination of the stimulus. In addition to these, we have observed a different type of response in neurons of the mouse inferior colliculus (IC). These responses had latencies of 100 msec to over 200 msec and required tone bursts of such relatively long durations in order to significantly respond. In a sample of more than 300 IC neurons of mice tranquilized with chlorprothixene and only minimal barbiturate, 8 units of this type were observed. None appeared to be located in the ventrolateral (central) nucleus. Since optimal responding required stimuli of particular minimal durations, these units appear to "filter" out short duration sounds. Such responses are fundamentally different from previously mentioned sustained and on-off responses which follow the temporal course of stimuli over wide duration ranges. For the units described here, relatively long duration is an obligatory stimulus "feature," and the very fact that a response occurs provides information about duration.

43 CHANGES IN ENDOGENOUS REACTIVITY TO DIAMINOBENZIDINE IN BRAIN STEM AUDITORY NUCLEI SUBSEQUENT TO THE "SILENCING" OF THE AUDITORY NERVE. Margaret Wong-Riley, Michael M. Merzenich* and Patricia A. Leake*. Dept. Anat. and Coleman Lab., Univ. Calif., San Francisco, Ca. 94143.

In previous studies (Wong-Riley, Brain Res., 108 (1976) 257-277, and unpublished observations), neurons and/or neuropil in various relay nuclei of the auditory pathway (cochlear nuclei, superior olivary nuclei, nuclei of the lateral lemnisci, inferior colliculi) were found to be highly reactive to the diaminobenzidine-H_2O_2 medium at pH 9.0. This was interpreted to be due largely to a higher concentration of heme-enzymes, most likely of the cytochrome system, within these regions. In order to test the possible relationship of such oxidative enzyme activity to the functional state of these nuclei, experiments were carried out in cats whose auditory nerves were unilaterally "silenced" for periods of 1-10 months. These cats (implanted with long silastic scala tympani inserts; Schindler and Merzenich, Ann. Otol. Rhinol. Laryngol., 83 (1974) 202-216) were totally deaf in one ear, while the auditory nerve fibers of that side were determined histologically to be largely intact. When the brain stems of these cats were reacted with the DAB-H_2O_2 medium, neurons or neuropil that received their major excitatory input directly or indirectly from the deaf ear exhibited decreased reactivity. Thus, the level of reactivity was significantly reduced within both neurons and neuropil of the ipsilateral anteroventral and posteroventral cochlear nuclei, and of the ipsilateral lateral superior olives (which receive excitatory input from the ipsilateral ear, and inhibitory input from the contralateral ear). Neurons of the contralateral medial nucleus of the trapezoid body, and neuropil of the contralateral ventral nucleus of the lateral lemniscus and at least the caudal (monaural) portion of the contralateral central nucleus of the inferior colliculus also exhibited markedly reduced reactivity. By comparison, there was relatively little difference in reactivity between the dorsal cochlear nuclei, the medial superior olives (which receive excitatory input from both ears) and the dorsal nuclei of the lateral lemnisci of the two sides.

The decrease in presumed oxidative enzyme activity within major auditory nuclei seen to the level of the midbrain in unilaterally deafened cats reflects a sensitive response of neurons to the elimination of their primary excitatory input(s) one to several synapses away.

(Supported by NIH Grants NS-12995, NS-11804 and NS-10414).

44 THE ANATOMIC AND ELECTRONIC FACTORS IN THE PRODUCTION OF COCHLEAR MICROPHONICS OR THE WEVER-BRAY PHENOMENON. M. Wharton Young*, Emeritus Professor of Anatomy, (SPON: James A. Holloway). Howard University, College of Medicine, Washington, D.C., 20059

When Wever and Bray first demonstrated the cochlear microphonic effects in 1930, they theorized that the anatomical basis for this physiological activity was "..a cochlear structure acting as a special receptor and transmitter" hence a true microphone. Numerous studies have reconfirmed these electrical effects, even in the post-mortem state but the anatomic and the electronic bases have remained somewhat obscure and speculative. The labyrinthine structure of the inner ear presents many gross and molecular problems. Throughout the prenatal period, the amniotic fluid exerts a constant and equal pressure on the developing organs which approximates that of the cerebrospinal fluid of the mother. After birth, the persons own cerebrospinal fluid pressure upon the endolymphatic sac is transmitted through its duct to balloon the membranous labyrinth and force (propel) its endolymph across the ultra thin vestibular (Reissner's) membrane into the perilymphatic space thus generating a streaming membrane potential, DC. The perilymph does not drain through its duct into the subarachnoid space but this duct ends in a terminal 'perilymphatic sac' similar to the endolymphatic duct and sac. This newly discovered sac lies in the jugular fossa above the carotid sheath. The perilymph makes its exit through the 'fistula ante fenestram' into the tympanic lymph channel that grooves the promontory and drains into the tubal tonsils. The retina and organ of Corti have a similar embryonic development and each individual hair cell develops a protoplasmic outgrowth (projection) of a different length that forms a dorsal cuticular border on Corti's organ that grows and expands to form the pennant-shaped tectorial membrane attached medially to the individual auditory (Huschke's) teeth on the limbic lip, adjacent to the highly charged vestibular membrane. Each individual hair cell with its outer tectorial segment and 'tooth' forms a 'cochlear rod' (as retinal rods) whose specific length renders it as a selective resonator sensitive and responsive to a particular sonic frequency (cps): like radio antennae. Chemicophysical experiments indicate that the cytoplasmic sound-sensitive macromolecules are helical in shape and linearly arranged hence harmonious sonic vibrations cause ionic derangements (migration) that generate displacement potentials, CM, that initiate the local nerve impulse, NP, which is relayed to definite brain centers and specific cortical areas (Place Theory). Obviously each one of the twenty some thousand 'cochlear rods' is needed "..a cochlear structure acting as a special receptor and transmitter" and is thus a transducer and microphone.

45 AUDITORY FEATURE DETECTORS IN THE CANARY FOREBRAIN AND THEIR RESPONSE TO SONG. Malcolm D. Zaretsky*. Dept. Zoology, Univ. of Iowa, Iowa City, IA 52242.

The auditory areas of the canary forebrain contains single units which respond to various optimal stimuli: pure tones over a narrow band, pure tones over a wide range, FM signals, clicks. There are phasic and tonic units, on units, off units and units which respond with long latency.

The song of the roller canary consists of several phrases in succession, each formed by repetition of a distinctive syllable. The spectral composition of each syllable can be analyzed by means of the sound spectrograph. Auditory units respond to elements of natural song which have spectral characteristics similar to those of artificial optimal stimuli.

AUTONOMIC FUNCTION

46 INCREASED DEPRESSOR RESPONSES TO CENTRALLY ADMINISTERED NOREPINEPHRINE & EPINEPHRINE IN SINOAORTIC DENERVATED RATS. Natalie Alexander* and Charles K. Haun* (SPON: Bertha Newman) Depts. of Med. and Anat., Sch. of Med., USC, Los Angeles, CA 90033.

Resection of carotid sinus and aortic depressor nerves in the rat produces moderate, labile hypertension associated with elevated plasma dopamine beta hydroxylase activity (Life Sci. 18:655, 1976). Intra-arterial pressure recordings from conscious sinoaortic denervated (SAD) rats show wide oscillations of 25-50 mm Hg lasting 2 to 5 min with superimposed large pressure dips of 1-30 sec duration. The same occurs in SAD rabbits (PSEBM 121: 766, 1966). We hypothesized that SAD not only eliminates a major source of neural inhibition to the cardiovascular system, thereby raising systemic pressure, but also causes a "central denervation supersensitivity" to neurotransmitters. To test this idea, a 23-gauge canula extending into the fourth cerebral ventricle was implanted 10-12 mm posterior to the bregma in adult rats. A week later SAD or a sham operation was performed. Four to 21 days later a femoral artery was canulated (under ether or Innovar) and 3 hours later with the rat sitting in a restrainer, arterial pressure (AP) and pulse rate (PR) were recorded continuously before and after intraventricular injections of 1,2,4,8 and 16 µg (base) of l-norepinephrine bitartrate (NE) or l-epinephrine bitartrate (E) dissolved in saline. Each dose was in a volume of 1 µl and injected at 10-15 min. intervals. Both amines caused reductions in AP and PR. In some studies a NE and E dose-response curve was obtained in the same rat with an interval of 2-18 hrs. between the 2 tests. If this was done with an interval of only 1 hr. between tests, responses to the second amine were clearly attenuated suggesting that NE and E occupy some of the same central receptor sites to produce AP lowering. Both NE & E caused greater reductions of AP in SAD rats than in shams so that dose-response curves of SAD rats were shifted to the left. In contrast, PR reductions in response to NE or E were the same in both groups. SAD rats also had greater AP reductions than shams under urethane anesthesia where starting pressures were equal. One interpretation of these results is that pressure lability and pressure dips in SAD rats are due to intermittent release of adrenergic amines acting on brain stem vasomotor neurones which are supersensitive to neurotransmitters following interruption of neural input from baroreceptor nerves. Neurons involved in reflexes that affect PR did not show this supersensitivity to NE or E.

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47 THE EFFECT OF GUANIDINE ON TRANSMISSION AT THE CHICK CILIARY GANGLION. Floyd W. Banks. Dept. of Physiol., Univ. of Colo. Sch. Med., Denver, CO 80262.

Guanidine hydrochloride (CH_5N_3-HCl) has been shown to increase the size of the end-plate potential at the neuromuscular junction and also to cause hyperexcitability in axons (Matthews, G., and Wickelgren, W.O. J. Physiol. 266, 69-89, 1977). The effect of guanidine was tested on the chick ciliary ganglion to see if the increase in transmitter release could be accounted for by an increase in amplitude or duration of the presynaptic action potential or whether the drug had an effect on the release mechanism itself. When 0.5 mM guanidine was perfused into the bath and a presynaptic terminal (calix) was impaled, the calix spike showed a transient increase in amplitude and broadening and, occasionally, subsequent failure. This failure was probably due to a measured decrease in input resistance of the calix which enhanced the impedance mismatch between the small presynaptic axon and the large caliciform terminal. When the ganglion cell was impaled and guanidine applied, there was an increase in average amplitude of evoked e.p.s.p.'s. To test if this increase was associated with the broadening of the presynaptic spike, only ganglion cells with coupling potentials were impaled. After recording control e.p.s.p.'s, guanidine (0.5 mM) was perfused into the bath. Records were taken one every 4 sec for at least 5 min. A graph of the average amplitudes of the electrical coupling potential and chemical e.p.s.p. showed that the increase in chemical transmission preceded any changes in electrical transmission by 2.0 - 2.5 min. The time course of the change of electrical transmission closely followed the time course of broadening of the presynaptic spike when the calix was impaled directly. Amplitude histograms were made of evoked responses in control solution and in guanidine. The mean amplitude of the responses was larger and the coefficient of variation smaller in the guanidine solution. The decrease in the coefficient of variation is consistent with the idea that the increase in amplitude was due to an increase in quantal content and not due to increased sensitivity of the postsynaptic membrane. Supported by NIH grant # NS 09660 and Fellowship # NS 05554.

48 TONIC SYMPATHOINHIBITION OF NON-BARORECEPTOR ORIGIN. Susan M. Barman and Gerard L. Gebber. Dept. of Pharmacology, Michigan State University, East Lansing, Michigan 48824.

Experiments were performed on Dial-urethane anesthetized cats to determine whether neural inhibition of non-baroreceptor origin functions tonically to depress naturally occurring sympathetic nervous discharge (SND). The effects on renal SND produced by bilateral electrolytic lesions (5 mA for 15 sec) of the nucleus of the tractus solitarius (NTS) and of medial medullary structures were compared in baroreceptor intact and denervated animals. In the intact animal, NTS lesions 1 mm rostral and 1 mm caudal to the obex produced a significant ($p<0.05$) increase in renal SND (154±13% of control) and abolished the baroreceptor reflex. Loss of the baroreceptor reflex was evidenced by the disruption of the phase relations between SND and the cardiac cycle. NTS lesions increased SND in only 1 of 5 cats in which the carotid sinus, aortic depressor, and vagus nerves were bilaterally sectioned. These results suggest that sympathoinhibitory elements within NTS usually are not tonically active in the baroreceptor denervated cat.

Lesions in the midline of the medulla 0-2 mm rostral to the obex produced a significant increase in renal SND (142±11% of control) without affecting the baroreceptor reflex in intact cats. These lesions destroyed portions of nucleus raphe obscurus and the paramedian reticular nucleus. Midline medullary lesions in the baroreceptor denervated cat produced an equivalent increase in renal SND (161±13% of control). Section of the spinal cord at the first cervical segment essentially eliminated renal SND after medial medullary lesions. These data support the contention that inhibition mediated through midline structures in the caudal medulla is tonically active in the absence of baroreceptor nerve discharge and functions to suppress SND emanating from supraspinal structures. Furthermore, sympathoinhibition mediated through midline medullary structures is transmitted over a pathway distinct from that which receives baroreceptor input.

In contrast to the results obtained in animals with an intact neuraxis, midline medullary lesions failed to produce an increase in renal SND in baroreceptor denervated cats which were decerebrated at the midcollicular level. These results suggest either that sympathoinhibition mediated through midline medullary structures is of forebrain origin or that the increase in SND produced by midline lesions in cats with an intact neuraxis emanates from forebrain structures. (Supported by PHS grant HL 13187 and by a grant from the Michigan Heart Association.)

49 EFFECT OF CLONIDINE AND CHLORPROMAZINE ON ELECTRODERMAL RESPONSES AND REFLEXES AND THEIR INTERACTION WITH YOHIMBINE. Patricia J. Bernthal* and Michael C. Koss. Dept. Pharmacology, Univ. Okla. Sch. Med., Okla. City, Oklahoma 73190.

Adult cats were anesthetized with either sodium pentobarbital (36 mg/kg) or α-chloralose and urethane (35 mg/kg and 500 mg/kg). Electrodermal responses were evoked by electrical stimulation of the posterior hypothalamus at a constant submaximal frequency (10-16 Hz). Electrodermal reflexes were elicited by stimulation of the afferent common peroneal nerve in intact and spinal preparations. Peripheral electrodermal responses were evoked by stimulation of the efferent ulnar nerve. Graded i.v. doses of both clonidine and chlorpromazine reduced the amplitude of the centrally evoked responses. The ED_{50} for clonidine was approximately 5 µg/kg and that for chlorpromazine was about 1 mg/kg. Yohimbine pretreatment (0.5 mg/kg) antagonized the effects of clonidine but did not alter the action of chlorpromazine in inhibiting these responses. Both clonidine and chlorpromazine also reduced the amplitude of the electrodermal reflexes in a dose-related fashion. The ED_{50} for clonidine was approximately 1 µg/kg in the intact preparations and 2 µg/kg in the spinal cats. The ED_{50} for chlorpromazine was about 0.1 mg/kg and 0.03 mg/kg respectively in these two types of preparations. As with the centrally evoked responses, pretreatment with yohimbine effectively blocked the actions of clonidine but not that of chlorpromazine. None of these agents had any significant effect on the peripherally evoked responses. The results demonstrate that both clonidine and chlorpromazine inhibit central reactivity of this sudomotor system and that they can exert their action at several levels including the spinal cord. In addition, the observation that yohimbine selectively blocks the action of clonidine but not chlorpromazine suggests that these two agents depress the central reactivity of this sympathetic-cholinergic system by different mechanisms. (Supported by USPHS grant MH 25792)

50 TRANSIENT LOCAL ALTERATIONS IN CEREBRAL MICROVASCULAR BLOOD VOLUMES AND FLOWS INDUCED BY HYPOTHALAMIC STIMULATION. Baruch Blum*, Tamar Yashin*, Villi Benari*, and Stephan Friedland*. (SPON: W. A. Alter III). Dept. Physiol. & Pharmacol., Tel Aviv University, Tel-Aviv, Israel.

Evidence has been accumulated for neurogenic control of regional cerebral blood (RCB) supply. This includes anatomical data on rich autonomic innervation of various components of leptomeningeal vasculature and pharmacological data suggestive of neural control. This report presents physiological evidence on fast local changes in RCB supply at middle and posterior syprasylvian gyri (SSG) following lateral hypothalamic (LH) stimulation. Data were obtained from 24 chloralose-anesthetized cats. Application was made of silicon semiconductor detector for measurement of radioactive krypton (Kr) clearances after its bolus injection into internal carotid artery. This permitted computation of baseline RCB flow during stationary conditions and also comparison between microvessel blood volumes in stationary vs dynamic states induced by LH stimulation. The Zierler stochastic method and compartmental analysis used gave values agreeing in the low and medium ranges but diverged in the high flow ranges. Compartmental analysis revealed two gray-matter compartments within SSG: a fast one with a low weight(average f_{fast} = 220 ml/100g/min and average W_{fast} = 16% and a slow one with a high weight(average f_{slow} = 37 ml/100g/min and average W_{slow} = 84%). The fast compartment showed greater response to LH stimulation. Heat-clearance method calibrated by simultaneously applied Kr clearance was used to measure flow for stationary control periods and for dynamic conditions induced by LH stimulation. This method proved very sensitive for measurements of small changes. It was thus established that LH stimulation induces local RCB supply changes--either increases or decreases--with flow rates and microvessel volumes changing even independently, at latencies often shorter than 1 second. Phenomena of adaptation, recruitment, and oscillation were also observed. Microscopic observations, aided sometimes by fluorescein, enabled locating sites of maximal effects and observation that LH stimulation induced opening of capillaries that were previously closed and of widening of microvessels that were only partially open. Based on the shortness of latencies for changes and specificity of stimulation site, it was concluded that the data support a neurogenic control mechanism for gray-matter RCB blood supply.

51 MODULATION OF SOMATO-VAGAL REFLEXES BY SEPTAL STIMULATION IN THE RABBIT. A. L. Brickman, G. J. Mogenson and F. R. Calaresu, Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

It has been suggested that limbic forebrain structures are involved in cardiovascular integration by altering mechanisms lower in the neuraxis so that cardiovascular adjustments are appropriate to sensory inputs (Calaresu, Faiers and Mogenson, Prog. Neurobiol. 5: 1-35, 1975). Since it is well documented that electrical stimulation of somatic afferents elicits cardiovascular responses (Sato and Schmidt, Physiol. Rev. 53: 916-947, 1973), a series of experiments was done to investigate the role of the septum in the cardiovascular response elicited during somatic nerve stimulation.

Electrical stimulation of histologically verified sites in the lateral septum consistently elicited decreases in heart rate (HR) and mean arterial pressure (MAP). These responses were observed in rabbits anesthetized with either sodium pentobarbital (22 sites) or alpha-chloralose (20 sites). They were not due to changes in somatic activity since all rabbits were paralysed with decamethonium bromide (4 mg/kg, i.v.) and artificially ventilated. Stimulation of the sciatic nerve in rabbits anesthetized with chloralose elicited an immediate and pronounced drop in HR as well as a decrease in MAP. The HR response was shown to be due to vagal excitation as it was abolished by either bilateral vagotomy (n=8) or the administration of atropine methylbromide (3 mg/kg, i.v.; n=4). In order to investigate the influence of septal stimulation on the sciatic response, the lateral septum and sciatic nerve were stimulated simultaneously. The current intensities used during simultaneous stimulation were determined by stimulating either the sciatic nerve or the lateral septum individually using the threshold stimulus for a cardiovascular response. Combined threshold stimulation elicited a significant decrease in both HR and MAP (n=8). These findings suggest that the septum may participate in modulating cardiovascular responses elicited during somatic nerve stimulation.

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52 MECHANISMS INVOLVED IN THE CARDIOVASCULAR AND BEHAVIORAL CHANGES EVOKED BY CHOLINERGIC STIMULATION OF THE POSTERIOR HYPOTHALAMUS. J. J. Buccafusco* and H. E. Brezenoff. Dept. Pharmacol. CMDNJ, New Jersey Medical School, Newark, N. J., 07103.

We have demonstrated previously that stimulation of cholinergic receptors specifically localized in the posterior hypothalamic nucleus (PH) via microinjection (0.5µl) of carbachol produces a dose-dependent increase in blood pressure and a marked increase in motor activity in conscious, freely-moving rats (Fed. Proc. 36: 950, 1977). Dose-related changes in heart rate accompanied the consistent pressor and behavioral responses, however, the direction of the change was variable; increases and decreases in heart rate occurring with equal probability. Prior microinjection of the muscarinic antagonist atropine (6 nmole) inhibited the hypertensive (+40±3 mmHg) and bradycardic (-57 ±5 beats min^{-1}) response to 3 nmole of carbachol injected into the same site. In contrast neither the tachycardia (+71±7 beats min^{-1}) nor the increase in motor activity (405±45 movements per 15 min) evoked by carbachol were affected by atropine. Prior microinjection of the nicotinic antagonist mecamylamine (6 nmole) produced no effect on the cardiovascular changes to 3 nmole of carbachol but significantly reduced the increase in activity. Atropine and mecamylamine produced no cardiovascular or behavioral changes of their own when injected into the PH.

In anesthetized rats intravenous injection of 2 mg/kg of phentolamine produced a 60% reduction in the pressor response to electrical stimulation of the PH. This dose of the α-adrenergic blocking agent administered i.v. to unanesthetized rats also reduced the pressor response to chemical stimulation of the PH with carbachol by a similar amount.

These results indicate that a cholinergic mechanism exists in the PH which mediates a rise in blood pressure and a fall in heart rate through muscarinic receptors; and an increase in motor activity through nicotinic receptors. The nature of the receptors mediating the tachycardic response to carbachol is unknown. The pressor response evoked by carbachol appears to be mediated at least in part through an increase in sympathetic nervous outflow.

53 SYMPATHOADRENAL MEDULLARY ACTIVITIES IN SPONTANEOUSLY HYPERTENSIVE RATS AND WISTAR KYOTO RATS FOLLOWING RESTING, HANDLING, RESTRAINT, COLD STRESS AND FOOT-SHOCK STRESS. C.C. Chiueh*, R. McCarty and I.J. Kopin. NIMH, Bethesda, Md. 20014.

Blood (0.5 ml) was obtained from undisturbed spontaneously hypertensive (SHR) and the normotensive Wistar Kyoto (WKY) rats through a chronic indwelling tail arterial cannula and assayed for plasma norepinephrine (NE) and epinephrine (EPI) by a modified radioenzymatic paper chromatographic method. The plasma levels of NE and EPI are a good index of the sympathoadrenal medullary activities because the increments of plasma catecholamines are found to be dependent upon the degree of stress (Chiueh et al., Pharmacologist 18:135, 1976). There was no significant difference in 'basal' plasma levels of NE and EPI in blood obtained from the conscious and undisturbed SHR and WKY rats 24-48 hours after cannulation despite the fact that blood pressures of SHR rats were 25-50 mmHg higher than those of WKY rats. Several experimental manipulations, i.e., transfer of rats into a new 20x40x40 cm^3 cage (handling), confinement of rats in a 6.5x6.5x20 cm^3 restrainer of the tail cuff procedure for indirect measurement of blood pressure (restraint) and cold stress at 4°C, increased plasma NE and EPI in both WKY and SHR rats. The increments in plasma NE and EPI in SHR rats were not significantly different from those of WKY rats during handling or after 20-240 min. at 4°C. However, there was a greater elevation of plasma catecholamines, mainly, EPI, in restrained SHR rats than in restrained WKY rats. In the restrainer, the blood pressures of SHR rats increased approximately 25 mmHg while those of WKY remained unchanged. Hyperadrenergic responsivity in SHR rats was also noted during and after 5 min. foot-shock stress (2mA, 0.1 Hz, 0.4 sec). During the 5 min. intermittent foot-shock period, a 4-7 fold increase in plasma levels of NE and EPI was found in WKY rats and an 8-21 fold increase in SHR rats. Fifteen minutes after the shocks, when the rats still remained in a freezing posture suggesting an emotional stage of fear of shock, elevated levels of EPI and NE were found in SHR rats but not in WKY rats. In conclusion, while there was no difference between WKY and SHR rats in basal sympathoadrenal medullary activities, hyperadrenergic responses were found in SHR rats during restraint, foot-shock and possible emotional stress but not during other stresses.

54 DO SOME CARDIOINHIBITORY AXONS ORIGINATE IN THE EXTERNAL CUNEATE NUCLEUS? J. Ciriello* and F. R. Calaresu, Dept. of Physiology, University of Western Ontario, London, Canada N6A 5C1.

The external cuneate nucleus (ECN) has been shown to receive an input from the aortic depressor nerve (Am. J. Physiol. 215: 269-276, 1968) and from a cardioacceleratory area in the posterior hypothalamus (Ciriello and Calaresu, in preparation). These findings suggest a role for this nucleus in central regulation of the cardiovascular system and have prompted the following experiments.

Electrical stimulation of 78 histologically verified sites in the ventral ECN in 15 chloralosed, paralyzed and artificially ventiled cats, consistently elicited a marked decrease in heart rate, with a threshold current of 5-25 μA and an optimal frequency of 20 Hz when using 0.2 ms pulses. This response was shown to be due to vagal excitation as it was not affected by propranolol (1.5 mg/kg, i.v.) but was abolished by ipsilateral vagotomy and by the administration of atropine methylbromide (2 mg/kg, i.v.). The possibility that the bradycardia observed was due to activation of cardiovascular afferents terminating at or near this nucleus was investigated in seven chloralosed cats by studying the effect of administration of progressively larger doses of pentobarbital, a barbiturate known to depress synaptic transmission, on the magnitude of the bradycardia elicited by stimulation of a known site of termination of baroreceptor afferents (n. tractus solitarius, NTS) and that elicited by stimulation of either a known site of origin of cardioinhibitory neurons (n. ambiguus, AMB) or the cervical vagus. Bradycardic responses to stimulation of the ECN, the AMB and the cervical vagus were of the same magnitude and were affected by the barbiturate significantly less than the response from the NTS, suggesting that the ECN may be a site of origin of cardioinhibitory axons. In six additional experiments the possibility that the bradycardia observed during ECN stimulation was due to activation of cardioinhibitory axons originating in the AMB was tested by making unilateral lesions of the AMB and, after sufficient time had passed for the fibers originating in the AMB to degenerate (11-23 days), the magnitude of the bradycardia elicited by stimulation of sites in the ECN ipsi- and contralateral to the lesion was compared. Stimulation of the ECN on either side in these animals still elicited a marked bradycardia which was abolished by cutting the ipsilateral cervical vagus. These results suggest that the ECN is a site of origin of cardioinhibitory axons in the cat and must therefore be considered an important component of reflex arcs leading to cardiac slowing.

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55 EFFECT OF BIOFEEDBACK INDUCED CHANGES IN SKIN TEMPERATURE ON NON-INVASIVE REGIONAL CEREBRAL BLOOD FLOW IN NORMAL SUBJECTS. James L. Claghorn*, Roy J. Mathew*, John W. Largen*, John S. Meyer* (SPON: Beng T. Ho). TRIMS, Houston, Tx. 77030

A study to ascertain the effects of biofeedback induced skin temperature changes on regional cerebral blood flow was undertaken at the Texas Research Institute of Mental Sciences, in accordance with the hypothesis that such techniques may be utilized in the treatment of migraine headache syndrome. Migraine has been characterized as a stress related syndrome, with painful cephalic dilation during the headache phase which may be precipitated by excessive sympathetic activity; hand warming through biofeedback temperature training may result in a decreased sympathetic outflow to the cephalic vessels, an alteration in the vasomotor state, and a consequent abortion of the headache phase.

Subjects are 12 normal female volunteers, aged 19-35, randomly assigned to either a hand warming or hand cooling temperature feedback group. Each participant was trained to elevate or lower her hand temperature in a series of 9 biofeedback sessions, over a period of approximately 30 days. At the conclusion of training, all subjects underwent 2 measurements of non-invasive regional cerebral blood flow(nrCBF) via the ^{133}Xenon inhalation technique(pre and post skin temperature manipulation); this phase of the research was conducted in a carefully controlled laboratory setting.

The authors will present data comparing regional intracerebral and extracerebral blood flow measures at pre and post skin temperature manipulation; comparison between subject groups (hand warming or hand cooling) will be made, and correlations of change in nrCBF parameters with biofeedback induced skin temperature changes will be offered.

56 EFFECTS OF INDORAMIN ON CENTRAL SYMPATHETIC REACTIVITY. Meredith A. Davison and Michael C. Koss. Medical Biology Division, Okla. College of Osteopathic Medicine, Tulsa, Ok 74101.

Indoramin has been shown to reduce blood pressure in both humans and experimental animals (Royds et al., Clin. Pharmacol. Therap. 13: 380, 1972; Baum and Shropshire, European J. Pharmacol. 32: 30, 1975). Many features of the complex pharmacological profile of this agent suggest that its antihypertensive effect may be due in part to central actions (Baum and Shropshire, European J. Pharmacol. 32: 30, 1975). We have previously reported that the sympathetic-cholinergic electrodermal response (EDR) is an effective model system for studying the effects of drugs on central sympathetic reactivity, and that clonidine inhibits central reactivity in this system in a manner analagous to its action on other sympathetic systems (European J. Pharmacol. 37: 71, 1976). The present experiments used the electrodermal response system to investigate the possible action of indoramin on central autonomic activity. In anesthetized cats (pentobarbital, 36 mg/kg, ip) EDR were evoked centrally by stimulation of reactive loci in the posterior hypothalamus and peripherally by stimulation of the distal portion of the sectioned ulnar nerve. Indoramin (0.33-10 mg/kg, iv) significantly reduced the amplitude of the EDR evoked by stimulation of the hypothalamus at a constant submaximal frequency (10-16 Hz) is a dose-related manner. Indoramin had no effect on the peripherally evoked responses. As has been previously reported indoramin also caused a similar dose-dependent reduction in both blood pressure and heart rate. Unlike clonidine, indoramin did not induce mydriasis. Preliminary results indicate that the effects of indoramin on the EDR appear to be at least partially antagonized by yohimbine pretreatment (0.5 mg/kg, iv). The results of these investigations suggest that indoramin reduces central sympathetic reactivity elicited by hypothalamic stimulation.

57 EFFECTS OF TRH AND SOMATOSTATIN ON CENTRAL REGULATION OF BLOOD PRESSURE. Bernard Delbarre*, Danielle Senon* and Henry Schmitt* (SPON: Marthe Cohn). Lab. Ch. Exp. Fac. Med. 37032 Tours Cedex, Dep. Pharm. Broussais Hotel-Dieu Paris, France.

Thyrotropin releasing hormone (TRH) has been shown to be widely distributed in the central nervous system and has been proposed as a neurotransmitter. The effects of this agent on blood pressure and heart rate has been looked for after intracerebroventricular administration. Cats of either sex were anaesthetized with chloralose. Blood pressure, heart rate and splanchnic discharges were recorded. The injection of TRH (1 $\mu g/kg^{-1}$) into the lateral ventricle or the third ventricle of the brain induced a significant and long lasting increase in blood pressure, heart rate and splanchnic discharges. Similar results have been shown in pentobarbitalized dogs. Injection of TRH into the cisterna magna in the same dose, has no effect. Somatostatin (1 $\mu g/kg^{-1}$) injected by the same route in cats induced a slight but significant decrease in blood pressure and in addition antagonized the effects of TRH when injected before or after the latter hormone. TRH has been shown to activate the adenylate cyclase and somatostatin to reduce the level of adenosine 3':5' monophosphate (cyclic AMP) in the brain. As cyclic AMP has been shown to increase blood pressure after intracerebroventricular administration (Delbarre and all., Abst. 6 Int. Congr. Pharmacol 227, 1975-Clin. Exp. Pharm. Phys., 1977. In press) there is a possibility that cyclic AMP might be involved in the effect of TRH and somatostatin.

58 LATERALIZATION OF PRESSOR AND CARDIOACCELERATORY RESPONSES IN THE ZONA INTERMEDIA OF THE CAT SPINAL CORD. A. I. Faden*, M. Woods*, T. P. Jacobs* and C. F. Tyner (SPON: F. J. Manning). Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012.

Pressor and cardioacceleratory responses to electrical stimulation of the thoracolumbar spinal cord were studied in 19 anesthetized, vagotomized, paralyzed cats following low cervical spinal cord transection. Arterial pH, arterial blood gases and rectal temperature were closely monitored and maintained within normal limits. Pressor and cardioacceleratory sites were mapped using trains of constant current and stimulating at 0.25 mm intervals throughout the spinal cord.

Blood pressure and heart rate responses were very well localized within the zona intermedia and could be obtained with currents as low as 40 microamperes. Maximal response sites were demonstrated histologically to correspond to the intermediolateral nucleus. Both pressor and cardioacceleratory responses could be elicited throughout the longitudinal extent of the intermediolateral nuclear column (T_1-L_4), with the largest responses found in the upper thoracic region. The lumbar cardioacceleratory responses could be obtained after bilateral transection of the sympathetic chain at T_{11} but were abolished by spinal cord transection at T_{11}. Lumbar pressor responses could still be generated after T_{11} spinal cord transection, but were substantially reduced in magnitude. Cardioacceleratory and pressor responses were remarkably well lateralized at all levels of the spinal cord between T_1 and L_4, with significantly greater changes in heart rate and blood pressure obtained from right intermediolateral column stimulation as compared to the left. Change of anesthetic agent (alpha chloralose, sodium pentobarbital) and adrenalectomy failed to alter this lateralized responsiveness.

59 RELATION BETWEEN EXPIRATORY DURATION AND ROSTRAL MEDULLARY EXPIRATORY NEURONAL DISCHARGE. J.L. Feldman and M.I. Cohen, Dept. Physiol., Albert Einstein Col. Med., Bronx, N.Y.

In order to understand the central neural events that control expiratory duration (T_E), the responses of expiratory (E) neurons in the rostral medulla to various types of cycle triggered lung inflations were studied. Experiments were performed on decerebrate cats, gallamine paralyzed, with pneumothorax, and ventilated via a tracheal cannula with a cycle triggered pump (CTP; Feldman et al., Brain Res. 104, 341, 1976). The CTP inflated the lungs coincidentally with the period of the phrenic nerve burst, which served as indicator of the central inspiratory (I) phase. E unit activity was recorded with glass microelectrodes in the region of the nucleus of the solitary tract (NTS) and in the rostral part of the nucleus retroambigualis (rNRA). Changes in T_E were produced either by withholding inflations during the I phase or by applying maintained or phasic inflations during the E phase. Two major types of E neurons were observed: 1) Early expiratory: These began firing immediately after I cutoff, reached peak frequency shortly thereafter, and then declined in frequency throughout the remainder of the E phase. During the lengthened E phase which followed the lengthened I phase due to withholding inflation (Breuer-Hering I-inhibitory reflex), these neurons had a higher initial frequency and their discharge decayed more slowly. E inflations produced increased firing of these neurons together with lengthening of the E phase (Breuer-Hering E-facilitatory reflex). Many of these neurons were located in the NTS, which is thought to be a prime component of the I pattern generator. The responses of these neurons suggest that they control T_E by exerting inhibitory actions on I-facilitatory systems. 2) Late expiratory: These neurons, found predominantly in the rNRA, began firing with different delays after I cutoff, gradually increased their firing throughout the E phase, and stopped firing near the time of onset of the I phase. Withholding inflation did not markedly affect the initial discharge of these neurons but caused a prolongation of firing. In contrast, E inflations produced a marked inhibition of their discharge. These neurons could be involved in either: a) the control of the E musculature; or b) the control of T_E, by promoting the onset of the next I phase. (Supported by USPHS Grant HL-20800.)

60 CONVERGENCE OF INPUTS ONTO INTERNEURONS OF BARORECEPTOR REFLEX ARC. Gerard L. Gebber, Robert B. McCall and Susan M. Barman. Dept. Pharmacol., Michigan State Univ., East Lansing, MI 48824.

In a previous report from this laboratory (McCall and Gebber, Fed. Proc. 36: 488Abs., 1977) neurons whose discharges were interrupted within a heart beat upon bilateral occlusion of the common carotid arteries (BLCO) in vagotomized cats were located in the nucleus of the tractus solitarius (NTS), and in the vicinity of the intermediomedial spinal nucleus (IMM). These neurons were presumed to be interneurons in the baroreceptor reflex arc and additional evidence was offered that the units in IMM mediated spinal sympathoinhibition. In the present investigation, the spontaneous discharge patterns of BLCO-sensitive neurons in NTS and IMM of vagotomized cats were analyzed by constructing post-R wave time interval histograms (post-R wave TIH's). Six units in NTS exhibited unimodal histograms with peak probability of discharge occurring 70-80 msec after the R wave. The timing of this peak is consistent with the possibility that rhythmically-active input to these neurons was solely of carotid sinus baroreceptor origin. In this regard, the pulse synchronous component of carotid sinus nerve discharge began 68-71 msec after the R wave. The post-R wave TIH's of other units (n=11) in NTS and in IMM were multimodal. The timing of the second peak (70-80 msec), but not that of the earlier or later peaks, in the multimodal histograms was consistent with that of pulse synchronous carotid sinus baroreceptor discharge. Since the vagus and aortic depressor nerves were sectioned in these experiments, it is concluded that medullary and spinal interneurons of the baroreceptor reflex arc receive auxiliary afferent input from sources in addition to the IX and X cranial nerves. These auxiliary inputs apparently are responsible for the early (<50 msec) and late (>100 msec) peaks in the multimodal post-R wave TIH's of unitary discharge.

One source of auxiliary input is provided by sympathetic afferents in the inferior cardiac nerve. Single shocks applied to the inferior cardiac nerve elicited early and late orthodromic discharges in IMM neurons which exhibited multimodal post-R wave TIH's. The latency of the early discharge (5 msec) was shorter than that (8 msec) for activation of IMM units by NTS stimulation. This observation suggests that the early discharge was mediated over a spinal pathway. The latency of the late discharge (12 msec) of IMM neurons was consistent with activation over a supraspinal reflex pathway. Indeed, NTS units exhibiting multimodal post-R wave TIH's were activated with a latency of 6 msec by single shock stimulation of the inferior cardiac nerve. (Supported by PHS Grant HL 13187 and a grant from the Michigan Heart Association.)

61 EFFECTS OF PULMONARY AFFERENTS ON RESPIRATORY MODULATION OF SYMPATHETIC DISCHARGE. P.M. Gootman, J.L. Feldman, and M.I. Cohen. Depts. Physiol., Downstate Med. Ctr., SUNY, Brooklyn and Albert Einstein Col. Med., Bronx, N.Y.

Experiments were performed on decerebrate or urethane-anesthetized, gallamine-paralyzed cats with pneumothorax and intact vagi. Efferent splanchnic and cervical sympathetic nerve discharges were recorded monophasically (0.2-1000 Hz). Phrenic (PHR) nerve discharge served as an indicator of respiratory center output. Lung inflation was applied coincidentally with PHR discharge during control cycles by means of a cycle triggered pump (CTP; Feldman et al., Brain Res., 104: 341, 1976). Changes in the timing of CTP inflations were used to evaluate effects of pulmonary afferent (PA) activity from lung stretch receptors on central respiratory modulation of sympathetic (SYMP) discharge (Cohen and Gootman, Am. J. Physiol., 218: 1092, 1970). When inflation was not applied for one inspiratory (I) phase, the Breuer-Hering I-inhibitory reflex occurred: I prolongation with no change in slope of the integral of PHR activity, as measured with an average-response computer. In contrast this test produced an increase in slope of the integrated SYMP discharge. This indicates that inflations during the control I phases inhibited SYMP discharge. The striking difference in the slope changes of PHR vs. SYMP activities implies that this inhibition of SYMP activity was acting via circuits different from but related to those of the Breuer-Hering reflex. Lung inflation also inhibited SYMP discharge during the expiratory (E) phase, since inflations applied during the E phase reduced SYMP discharge, concomitantly with lengthening of the E phase (Breuer-Hering E-facilitatory reflex). The latency for SYMP inhibition from the onset of inflation was of the order of 100-200 msec for both I and E inflations. Vagotomy abolished these effects and resulted in an increased respiratory modulation of SYMP discharge. These results indicate that PA activity exerts an important influence on the central respiratory modulation of SYMP discharge. (Supported in part by NIH grants HL-20800 and NS-12031.)

62 EFFECTS OF DRUGS WHICH PRODUCE SYMPATHECTOMY ON THE RETROGRADE TRANSPORT AND ACCUMULATION OF NGF. Eugene M. Johnson, Jr., Roger Y. Andres, Richard A. Macia and Ralph A. Bradshaw. Depts. of Pharmacology and Biochemistry, Washington U. Med. School, St. Louis, MO 63110 (Spon: J. A. Ferrendelli).

Work by Hendry, Thoenen and co-workers has shown that nerve growth factor (NGF) is transported from the periphery to the cell body of sympathetic neurons by a retrograde axoplasmic flow. Since the cytotoxicity of drugs which destroy sympathetic neurons (6-hydroxydopamine, guanethidine, vinblastine) can be suppressed by concomitant administration of NGF, we have explored the possibility that these agents may all ultimately kill the cell by a common mechanism: prevention of the retrograde transport, accumation or utilization of NGF. Administration of high specific activity NGF (6-10 ng, ∿500,000 cpm) into the anterior eye chamber of adult rats resulted in a maximal accumulation (∿ 1000 cpm) of ^{125}I-NGF (confirmed by gel electrophoresis) in the ipsilateral superior cervical ganglion (SCG) about 16 hrs after injection. Very few counts (<30) were found in the contralateral ganglion. This system was "half-saturated" when 30-40 ng of NGF were injected into the eye chamber. Administration of NGF (200ng) systemically to adult (i.v.) or 5 day old animals (s.c.) resulted in maximal accumulation in SCG in about 8 hours. Administration of guanethidine (50 mg/kg/day i.p.) to adult rats resulted in a 50-60% decrease in accumulated NGF within 3 days of starting treatment. A single injection of 6-hydroxydopamine (100 mg/kg s.c.) or vinblastine (0.4 mg/kg s.c.) 16 hours prior to systemic administration of ^{125}I-NGF to 5 day-old rats prevented the accumulation of radioactivity in SCG. Administration of 6-hydroxydopamine to adult rats did not effect the accumulation of NGF during the first 6 hours after ^{125}I-NGF administration (i.v.), but accumulation ceased at that time rather than continuing for another 3 hours as in controls. These results support the notion that, although their initial interaction with the neuron may be very different, all of the drugs ultimately kill sympathetic neurons by "depriving" them of NGF. (Supported by Nat. Fdn., March of Dimes, NIH Grant HL20604 and NS10229, R. Y. A. is a fellow of the Swiss National Foundation.)

63 Gastric secretion and hyperglycemia provoked by cytoglucopenia in the nuclei of the solitary tract. M. Kadekaro, C. Timo-Iaria and M.L. Mendes Vicentini. Dept. Physiology and Pharmacology, Inst. Biomedical Sciences, Univ. of Sao Paulo, Cx. Postal 4365, Sao Paulo, S.P., Brazil.

Previous reports showed that cytoglucopenia induced by intravenous injection of 2-deoxy-D-glucose (2-DG) in cats increases gastric secretion by activation of several parallel mechanisms (J. Physiol. 1972, 221:1-13; J. Physiol. 1975, 252:565-584; Nerves and Gut, ed. F.P. Brooks, 1977, in press). 1. The first mechanism involves a neuronal chain whose afferent and efferent limbs are in the medial forebrain bundle (MFB) area; 2. The second circuit consists of glucoreceptors in the MFB area with the efferent limb in the globus pallidus; 3. The third reflex arc involves afferent pathways arising in the liver and efferent pathways in the lower brain stem; 4. The fourth loop is mediated by afferent and efferent pathways located in the lower brain stem. This latter circuit was first disclosed by injection of 2-DG (60 mg/kg) in the left vertebral artery of cats previously subjected to midcollicular transection of the brain stem and also in cats subjected to pontomedullary electrolytic transection. The present work shows that rhombencephalic receptors located in nuclei of the solitary tract are responsible for gastrosecretory and hyperglycemic responses to local cytoglucopenia but that gastrosecretory responses produced by microinjection into vagal nuclei is nonspecific. The identification of the rhombencephalic glucoreceptors was accomplished through microinjection of 3.3 µl/min of 2-DG (50 mg/ml) by means of a glass micropipette in several medullary structures of cats with midcollicular transection of the brain stem. At the end of the experiment a very low anodal current (10 µA x 10 sec) was passed through a microelectrode carried by the micropipette and the histological identification of the lesion (Prussian blue reaction) enabled us to locate the site of microinjection. Local microinjection of 2-DG within the boundaries of the nuclei of the solitary tract caused intense gastric secretion. Saline microinjection in the same areas failed to increase gastric secretion, showing that the effect is specific to glucorecepetor activation. Injection of either 2-DG or saline in the dorsal nucleus of the vagus caused an increase in gastric secretion which was apparently due to the mechanical stimulation of the nucleus. No secretory response was obtained by microinjection of 2-DG or saline into surrounding areas. Activation of the glucoreceptors involved in the gastrosecretory effect of cytoglucopenia also provoked a hyperglycemic response.
Supported by the Sao Paulo State Research Foundation (FAPESP).

64 NATURAL SUPPRESSION OF FEVER IN THE NEAR-TERM EWE: A CENTRAL PHENOMENON? Norman K. Kasting*, Warren L. Veale, Keith E. Cooper, Division of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

The production of fever involves an interaction between an exogenous pyrogenic factor (bacterial pyrogen - BP) and cells of the reticulo-endothelial system to elaborate endogenous pyrogen (EP). It is thought that EP enters the central nervous system to initiate responses leading to fever. It is well-known that the newborn human often does not become febrile even though it may be seriously infected. We have shown that the newborn lamb does not respond to either EP or BP immediately after birth. In our efforts to obtain more information regarding this phenomenon, we have examined the ability of the pregnant ewe near term to become febrile. Twenty-two pregnant ewes were injected intravenously with a bacterial pyrogen derived from Salmonella abortus equi (SAEP) several days before and immediately following parturition. The most commonly injected dose of SAE was 30 µg, an amount sufficient to produce a fever of 1.3 $\pm$0.15°C in a non-pregnant adult sheep. Endogenous pyrogen was made in vitro by incubating peritoneal exudate macrophages with SAEP. Amounts of EP were injected which produced a fever of 1.85 $\pm$0.10°C in the non-pregnant adult sheep. During these experiments rectal temperature was monitored continuously and the general physiological state of the animal was assessed.

The febrile response to SAEP was diminished or absent from 4 days prior to and 1 day after parturition. Similarly, the animals failed to become febrile following intravenous injection of EP over a similar time course. Since fever to both EP and BP are inhibited, it is suggested that this phenomenon is not associated with the elaboration of endogenous pyrogen by cells of the reticulo-endothelial system, but is more likely to be an inhibition of the action of EP on the central nervous system.

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65 SENSORY AND MOTOR PATHWAYS OF THE PULMONARY VAGUS: SIMULTANEOUS VISUALIZATION BY HRP TRANSPORT. David M. Katz* (Spon: A.D. Rosen) and Harvey J. Karten, Depts. of Biology (DMK) and Psychiatry (HJK) S.U.N.Y., Stony Brook, N.Y. 11794.

The dorsal motor nucleus of the vagus nerve (DMN) contains cells of origin of vagal efferent fibers in both birds and mammals. Nucleus solitarius (NS), which lies immediately lateral and dorsal to DMN in the floor of the fourth ventricle, receives sensory input from vagal afferent fibers. Numerous subdivisions have been recognized within both nuclei, but the relationship of those subdivisions to the individual peripheral structures innervated by the vagus is unknown. Horseradish peroxidase (HRP) labelling techniques were used in the pigeon to determine the topographic representation of the pulmonary branch of the vagus in both DMN and NS.

An HRP-filled cuff was placed on the cut end of the right pulmonary vagal branch in otherwise intact animals. Following various survival times HRP granules were observed in 1) cell bodies in the nodose and jugular-superior ganglia, 2) the tractus solitarius (TS), 3) the neuropil of the posterior ventrolateral nucleus of NS, parasolitarius lateralis (Pl) and 4) DMN and the ventral motor nucleus of the vagus (VMN). Labelled cells were found in several subdivisions of DMN, ipsilateral to the transected nerve, and were most heavily concentrated in a ventrolateral subdivision of the caudal portion of the nucleus. Bilateral labelling was observed in both Pl and TS. The rostro-caudal extent of labelling in Pl corresponded to the rostro-caudal levels at which labelled cells were found in DMN.

The distribution of HRP granules in the neuropil of Pl correlates well with the recent finding in mammals that Pl contains respiratory neurons receiving a vagal afferent input (ref.: von Euler, Br.Res., 1973). HRP appears to have been transported anterograde from nodose and/or jugular-superior ganglion cells whose central processes terminate in Pl, and whose peripheral processes extend into the pulmonary vagus. Pl was not labelled in similar experiments involving other vagal branches. These findings suggest that 1) pulmonary vagal afferents project centrally to the lateral parasolitary subdivision of NS; 2) the cells of origin of pulmonary vagal efferent fibers lie in several subdivisions of DMN, corresponding, perhaps, to different effectors within the lung receiving a vagal input and 3) the pulmonary vagus is generally represented at corresponding rostro-caudal levels in both DMN and NS.

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66 ROLE OF SUBTHALAMUS IN MEDIATION OF BRADYCARDIA RESPONSES IN RABBITS. Marc P. Kaufman, Robert B. Hamilton*, Guy K. Petrik* and Neil Schneiderman. Dept. Psychol., Univ. of Miami, Coral Gables, FL 33124.

In barbiturate anesthetized rabbits electrical stimulation (100 pulses/sec, 0.5 msec pulse-width, 10 sec train, <250μA) of 20 sites in the lateral zona incerta of the subthalamus (LZI) elicited bradycardia (M=94 bpm, SE=±8 from a baseline of 220 bpm) and a small decrease in mean arterial pressure (M=6 mmHg; SE=±2 from a baseline of 80 mmHg). In contrast, comparable stimulation of 17 sites in the medial zona incerta and H fields of Forel in the subthalamus evoked a small bradycardia response (M=-16 bpm, SE=±8) and an increase in mean pressure (M=17 mmHg, SE=±2). Bilateral section of the vagus nerves converted the bradycardia response to a slight heart rate increase and transformed the vasodepressor response into a small pressor response. Low frequency (25 pulses/sec) stimulation of LZI attenuated the bradycardia response (-94 vs. -13 bpm). Extracellular recordings from 7 units in the vicinity of the bradycardia responsive area of the LZI indicated that they received barosensory input. Onset latency for these 7 cells to single-pulse stimulation of the aortic nerve averaged 68 msec (SE=±20).

Fifteen neurons were observed in the nucleus of the tractus solitarius or in the dorsal vagal nucleus that were activated by single-pulse stimulation of sites in LZI previously shown to elicit bradycardia to train stimulation. The units were also activated by either single-pulse stimulation of aortic nerve, or were found to fire in synchrony with the EKG. In addition, 3 of the 15 cells were also antidromically invaded from the cervical vagus nerve and were therefore preganglionic and probably cardioinhibitory. Onset and peak latencies of the responses to LZI stimulation averaged 6 msec (SE=±.5) and 9 msec (SE=±.9), respectively. When the LZI was stimulated with a train of 10 pulses at a frequency of 100 pps, the probability of discharge was greatly increased as compared to single-pulse stimulation.

The results suggest that (a) train stimulation of LZI elicits primary bradycardia accompanied by a secondary fall in blood pressure, (b) neurons within the LZI receive barosensory input, (c) LZI neurons project with short latency onto cardioinhibitory neurons in the dorsal medulla, and (d) the discharge rate of these neurons is importantly influenced by temporal summation (Supported by NSF Grant BMS 75-10967 and by a grant from the Heart Association of Greater Miami).

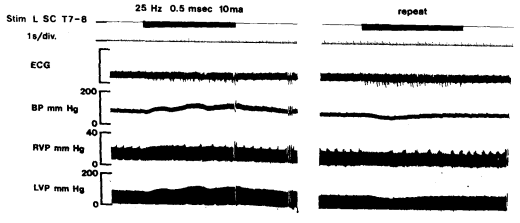
67 NEUROANATOMICAL SUBSTRUCTURE OF MEDULLAR RESPIRATORY NEURONS. Gary W. King* and Charles K. Knox. Laboratory of Neurophysiology, Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

Neuroanatomical tracing studies were performed in cats, utilizing retrograde axonal transport of horseradish peroxidase (HRP) and anterograde axonal transport of 3H-amino acids to delineate the medullar-pontine pathways involved in the neural control of respiration. The results show that two major groups of neurons in the medulla project to the parabrachial nucleus (NPB) of the dorsolateral rostral pons: 1) the medial nucleus of the solitary tract and dorsal motor nucleus of the vagus, which project topographically to NPB, and 2) lateral tegmental field (FTL) neurons. The FTL neurons are arranged in four longitudinal sheets which, on a frontal section, appear as groups of cells aligned radially with respect to the fourth ventricle. The sheets of FTL neurons are about 100μ thick and lie at the level of the obex near nucleus ambiguus, about 0.5mm apart. Furthermore, microscopic examination has revealed four longitudinal sheets of arterioles which coincide with the sheets of neurons projecting to the pontine respiratory areas. The neurons are thus interdigitated and closely juxtaposed to the arterioles. The most dorsal of the arteriolar sheets corresponds to nucleus retroambigualis, and extends from the spinal cord level C1, through n. ambiguus, and beyond the dorsal aspect of the seventh cranial nerve nucleus (c.f. Merrill, Brain Res., 24:11, 1970).

Preliminary electrophysiological studies have shown that respiratory-modulated neurons of the FTL are also found preferentially in these four sheets. Thus, the vascular system would appear to determine a topographical organization for respiratory neurons in the medulla. Also, if central chemosensitivity is located in deeper FTL regions (Lipscomb and Boyarsky, Resp. Physiol., 16:362, 1972), then these arteriolar sheets would provide a rapid sampling of arterial blood for the chemoreceptors.

68 SYMPATHETIC DEPRESSOR REFLEX ELICITED BY STIMULATING THE SYMPATHETIC CHAIN IN THE CANINE AND PRIMATE. David R. Kostreva, Fran A. Hopp* and John P. Kampine*. Depts. Physiol. and Anesthesiol., Med. College of Wisconsin and Wood VA Ctr., Milwaukee, WI 53193

Electrical stimulation of the left or right sympathetic chains (SC) after sectioning of the ventral and dorsal ansae subclavia and the SC below T12 resulted in a depressor reflex response in the canine and primate. Mongrel dogs and pigtail macaque monkeys (*Macaca nemestrina*) were anesthetized using phencyclidine hydrochloride, 2 mg/kg i.m., and sodium pentobarbital, 35 mg/kg i.v. The animals were placed on positive pressure ventilation, and the second through seventh ribs were removed from the left and right sides along with the adjacent sternum to allow unimpeded access to both SC. Right ventricular pressure (RVP), systemic arterial blood pressure (BP), and left ventricular pressure (LVP) were monitored from a femoral vein and arteries, respectively. The electrocardiogram (ECG) was monitored from leads placed in a lead II configuration. A representative response to stimulation of the left SC after section of the left ventral and dorsal ansae subclavia is shown in the left panel of the figure. With stimu-



lation of the left SC between T7 and 8, increases in BP, RVP and LVP were observed. However, when the left SC was sectioned at T12 and the SC stimulation repeated, as shown in the panel on the right, BP and LVP decreased with little or no change in RVP. Similar responses were observed when the upper (T1 or 2) and lower segments (T11 or 12) were stimulated on either the right or left SC in both the canine and primate. This study has provided some evidence for the existence of a sympathetic depressor reflex that can be elicited by stimulation of thoracic and abdominal sympathetic afferents.

69 NEURAL RELATIONSHIPS BETWEEN THE CELIAC PLEXUS AND COLONIC MOTILITY IN THE GUINEA PIG. David L. Kreulen* and Joseph H. Szurszewski. Dept. Physiol., Mayo Med. Sch., Rochester, MN 55901.

Experiments were performed to determine the relationship between synaptic input to neurons in the celiac plexus and colonic motility. In vitro preparations were dissected from guinea pigs and consisted of the celiac plexus attached to the whole colon by its vascular supply and nerve trunks. Intracellular electrical activity was recorded and nerve trunks were stimulated with external electrodes. Repetitive stimulation (5-15 Hz) of nerve trunks between the ganglia and the colon resulted in a 0.5 to 1.5 sec after-train hyperpolarization, a marked decrease in the level of synaptic input from receptors located in the colon and a decrease in colonic intraluminal pressure. In the superior mesenteric ganglion (SMG), only 5 of 77 neurons tested received synaptic input from a greater splanchnic nerve. In the celiac ganglia (CG), less than 10 percent of the neurons received synaptic input from both the ipsilateral greater splanchnic nerve and from the periphery. Thus in both the SMG and CG there was subganglionic organization of input. Neurons with a particular pattern of input appeared to be localized in specific regions. When the colon was connected to the ganglia 60 percent of the neurons tested in the SMG had continuous excitatory synaptic input. In the CG only 20 percent of the neurons received continuous excitatory synaptic input. In the neurons in both ganglia that did receive input, distention of the colon resulted in an increase in the level of synaptic input. However, all of these neurons did not receive synaptic input from all regions of the colon. For example, when the terminal colon was distended there was an increase in synaptic input to some neurons while there was no change in others. These results demonstrate subganglionic organization of neurons in the CG and SMG and that neurons in the CG and SMG receive mechanosensitive synaptic input from the colon. (Supported by Grants AM 17632 and T32 RL 7111-02)

70 EFFECT OF MESENCEPHALIC LESIONS ON THE CEREBROVASCULAR RESPONSE TO ELEVATED ARTERIAL PCO_2. J.H. Ovelmen Levitt and C.E. Rapela*. Depts. Neurology and Physiology-Pharmacology, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103

The CNS has been implicated by others as having a role in cerebrovascular control. The possibility that a mechanism located in the mesencephalon, influencing the cerebrovascular response to alterations in partial pressures of arterial carbon dioxide ($PaCO_2$) has been studied acutely in 22 dogs anesthetized with Chloralose. In 16 of these experiments midbrain lesions were made. Descending mechanisms influencing systemic blood pressure, ascending fibers from the locus coeruleus, the raphe nuclei, pathways associated with pain, as well as portions of the reticular activating system, and elements related to the limbic system are located in the midbrain and were involved in the lesions. In 6 animals no lesions were made in order to evaluate the effect of experimental time on sequential cerebrovascular responses to altered $PaCO_2$. During hypercapnia autoregulation by cerebral vessels is lost and cerebral blood flow (CBF) is highly dependent upon the level of the perfusion pressure. In order to separate the effect of lesions on the CBF from a concommitant effect on blood pressure, perfusion pressure-cerebral blood flow (PP-CBF) curves were obtained during normocapnia (N) and hypercapnia (H) twice during the control experiments; and in N and H before and after midbrain lesions in the lesion experiments. Cerebral PP changes were obtained by graded compression of the common carotids (CC) with the vertebral (V) arteries occluded. The mean of the CC pressure distal to the compression and the V wedged pressure was taken as cerebral PP. The BCF was recorded by the supratentorial venous outflow method. PP-CBF curves obtained before and after mesencephalic lesions, either during ($NPaCO_2$ = 35.1±5.25 mm Hg) or H($PaCO_2$ = 75.4±10.34 mm Hg) did not show significant differences. The mean post-lesion CBF response [CBF(H) - CBF(N)] was found to be 0.83 of the pre-lesion response (Conf. Lim. = 0.87-0.72). In the non-lesion control experiments, the second CBF response was 0.86 of the first (Conf. Lim. = 0.95-0.77). These CBF response ratio values for the lesion and control experiments were not significantly different. The results indicate that these midbrain lesions did not have a significant effect on the cerebrovascular response to increased $PaCO_2$ (75 mm Hg) within 60 minutes of lesion production. Supported by NIH Grants NS 11953, H1 5392, and NS 06655.

71 EFFERENT PROJECTIONS FROM NUCLEI OF THE SOLITARY TRACT IN THE CAT A.D. Loewy and H. Burton. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Previous studies have shown that afferent fibers from the cardiovascular, respiratory and other visceral systems terminate in the commissural (Com), medial (Sm) and ventrolateral (Svl) solitary nuclei. The solitary complex may be further subdivided to include a lateral (Sla) and an intermediate (Int) nucleus. The close proximity of these small nuclei and the heterogeneity of the cell types in some of them (e.g. Svl) make it difficult to analyze the efferent projections from this region with degeneration techniques.

By using retrograde and anterograde labeling techniques we have begun a systematic analysis of some efferent projections from the solitary complex in the cat. Small quantities of concentrated solutions of tritiated amino acids (50μCi/μl of leucine and proline) were injected into portions of the solitary complex through glass micropipettes. Our investigations have been confined to those regions from which activity related to respiratory or cardiovascular functions have been recorded. Following a survival of 7 days and subsequent autoradiographic processing, several of the efferent projections from the solitary complex have been observed. After an injection centered over Svl, descending fibers can be traced to the region of the phrenic motor neurons in the C5-C7 segments of the spinal cord and to the ventral tip of the thoracic ventral horn. Bilateral projections could be traced to the ventral and lateral funiculi surrounding the ventral horn. Ipsilateral projections were greater. Connections could also be traced in the medulla bilaterally to the retroambiguus, ambiguus, and retrofacial nuclei and contralaterally to the dorsal aspects of the paramedian reticular nucleus. Projections were traced to the ipsilateral medial accessory olive, rostral aspects of the dorsal motor nucleus of the vagus, contralateral Com, Sm, and Int nuclei of the solitary complex. Ascending connections were seen bilaterally in the medial and lateral parabrachial nuclei and Kölliker-Fuse nucleus.

The location of retrogradely labeled neurons in the solitary complex was also studied after injections of a 25% horseradish peroxidase solution. Following injections into the lower cervical and upper thoracic segments, medium sized neurons were labeled bilaterally in Svl, Sm and Int. HRP injections centered in medial accessory olive retrogradely label ipsilateral Sla, whereas, injections in the region of n. ambiguus label medium and large neurons in Svl. (Supported by USPHS Grants NS09809 and NS12751)

72 MEDULLARY AND SPINAL LATERAL HORN NEURONS RESPONSIVE TO STIMULI WHICH FORCE A CARDIOVASCULAR STATE-CHANGE. J. W. Manning and S. J. Putnam.* Dept. Physiol., Emory U., Atl., GA 30322.

Little or no information is available on the concurrent behabior of repetitively firing single units (RFNs) in the medullary reticular vasomotor area and in the intermediolateral cells column following stimulation of one or more of the following systems: anterior hypothalamus, posterior hypothalamus and carotid sinus nerve as well as stretch activation of baroreceptors by intravenous administration of noradrenaline (1μg/kg). Experiments were performed on ether induced, α-chloralose anesthetized cats paralyzed with Gallomine triethiodide; activity of brainstem medullary and lateral horn neurons was monitored extracellularly by tungsten μ-electrodes (7-11μF). Following suitable amplification arterial pulse pressure, ECG, unitary responses and respiratory event were stored on magnetic tape. The RFNs studied at the medullary level were in and around the stereotaxic coordinates P - 10, L-3, 2mm below floor of fourth ventricle, those in the lateral horn were at the T-1 level and identified as sympathetic preganglionic through antidromic activation by stimulation of the common vagosympathetic cervical trunk. The typical behavior of medullary RFNs has been reported previously; spinal RFNs do not fire with the characteristic burst pattern exemplified by many medullary units. The spontaneous activity of spinal units (ave. 6 per sec) has a lower frequency than that of medullary RFNs (ave. 13 per sec). Interspike interval analysis as well as post-stimulus histograms reveal a coupling of medullary and spinal unit response behavior. Correlation analysis will be sought between medullary and spinal RFNs with cardiac cycle. (Supported by NIH Grant HL 16648-13).

73 FUNCTIONAL CATEGORIES OF UNMYELINATED FIBERS IN RAT VENTRAL ROOTS. C.W. Maynard,* R.E. Coggeshall and T.B. Stubbs, III. Marine Biomedical Institute and Department of Anatomy, Univ. of Texas Medical Br., Galveston, Texas 77550.

We have previously identified large numbers of unmyelinated fibers in rat ventral roots T1-L2 and L6-S1, the same segments that contain large numbers of the smallest myelinated fibers. The present experiments are aimed at determining the functional categories of the unmyelinated axons in the L6 and S1 ventral roots. Ventral rhizotomies were performed on rats at the L6 or S1 segments. After three days the animals were sacrificed and the proximal and distal stumps of the cut root and the intact ventral root from the opposite side of the animal were taken for electron microscopic examination. To date, we have found in seven rats that the proximal stumps of the L6 or S1 ventral roots contain 70-80% as many unmyelinated axons as are found in the normal root. These are assumed to be unmyelinated preganglionic fibers. Thus 20-30% of the unmyelinated axons are missing from the proximal stump. We are in the process of determining the functional categories of these axons, one possibility being that they arise from dorsal root ganglion cells and are sensory in nature.

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74 ATTENUATION WITH ATROPINE IN THE BRAIN OF FEVER PRODUCED BY INTRA VENOUS PYROGEN: EFFECT OF SYMPATHECTOMY OF THE EARS OF THE RABBIT. B.J.C. Mutch*, K.E. Cooper and W.L. Veale, Division of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

Atropine injected into a lateral cerebral ventricle and the posterior hypothalamus of rabbits has been shown to attenuate fever produced by intravenous injection of pyrogen or intracranial prostaglandin E_1. Atropine has been postulated to block the febrile response by activation of heat loss mechanisms, such as vasodilatation and an increase in respiratory rate. The present investigation was undertaken to examine the effect of removal of sympathetic innervation from the ears of rabbits, and hence eliminate their ability to vasoconstrict, on fever production and its attenuation by intracerebral atropine.

Male New Zealand rabbits were anaesthetized, and fitted stereotaxically with headplates or cannulae, to serve as guides for microinjection into brain tissue or a lateral ventricle. In half of the animals the auricular nerves were isolated and cut. Several days later the animals were injected i.v. with endotoxin derived from Salmonella abortus equi (SAEP) and atropine sulphate was given in a lateral ventricle (200 µg/0.1 cc CSF) or in the posterior hypothalamus (50 µg/µl). Body temperature was measured by means of a rectal probe, and ear temperature was recorded using a disc thermistor attached to an ear.

For a non-denervated rabbit, ear temperature during a 1.5°C fever dropped from 36.5 ±1.5°C before the onset of fever, to 23 ±1.5°C just prior to the peak of the fever, at which point the ears rewarmed to their pre-fever temperature. However, for a rabbit with sympathectomized ears, the ear temperature accompanying a similar fever remained 35.5 ±2.0°C, without any correlation with stages of the fever. In the non-denervated rabbit, the ear temperature increased rapidly within 5 minutes following the injection of atropine to 37.0 ±1.5°C, and remained relatively constant for the duration of the action of the drug. When atropine was injected in conjunction with i.v. SAEP, the changes in ear temperature reflected the influence of both in relation to their relative time of injection. However, in denervated rabbits the ear temperature remained stable at 35.5 ±2.0°C throughout the experiment, apparently independent of heat loss or heat gain mechanisms which others have proposed to accompany fever.

It appears, therefore, that denervation of the ears of rabbits does not prevent fever, or the attenuation of fever by atropine.

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75 LOCALIZATION OF THE SACRAL AUTONOMIC (PARASYMPATHETIC) NUCLEUS IN THE SPINAL CORD OF CAT AND MONKEY BY THE HORSERADISH PEROXIDASE TECHNIQUE. I. Nadelhaft, C. Morgan, T. Schauble* and W.C. de Groat Dept. Neurosurg. & Pharmacol, Univ. of Pittsburgh School of Med. and Veterans Administration Hospital, Pittsburgh, PA 15261

A 25% aqueous solution of horseradish peroxidase (HRP) was applied to the central end of the transected whole pelvic nerve or to the branch going to the urinary bladder. In some experiments both nerves (bladder on one side, pelvic nerve on the other) were exposed to HRP. The animals were kept under anesthesia during the entire process which lasted between 24 and 48 hours, after which they were sacrificed and perfused with fixative. Frozen sections were processed using either diaminobenzidene or benzidene as the enzyme substrate, mounted, and examined under dark field illumination. The neurons belonging to the nucleus formed a long, narrow band lying mainly along the lateral edge of the grey matter. The highest concentration of labelled cells was in the second sacral segment but the longitudinal distribution had long tails on either side of the peak making its total length 7-8 mm. When the entire pelvic nerve was labelled, the cross-sectional aspect of the nucleus appeared as a band 100-150 microns wide extending for a length of about 1 mm from below the ventral extent of the central canal to just under the dorsal horn, at which point the band turned medially for about 300 microns. This medial extension was absent when only the bladder portion of the nerve was exposed to HRP. When diaminobenzidene was used, the cells and some of their dendritic extensions appeared with white granules in the dark field microscope. However, the benzidene substrate brought out additional features. In this case even the axons were visualized. These were seen to come from the ventral rootlets and pass near the edge of the ventral gray matter. The labelled cells were either spindle shaped (30-50 microns long, 12-15 microns wide) or multipolar (15-30 micron diameter). Frequently their dendrites could be followed for many cell widths in the 42 micron thick sections. These extensions for the most part followed the direction of the band (as seen in the cross section) but were sometimes observed to extend far into the white matter. The number of labelled cells varied in different cats from 700 and 1500. In the rhesus monkey preliminary results indicate that the nucleus is smaller in cross-sectional size (200 micron diameter) and much longer (13 mm) than in the cat. It is located at the lateral edge of the grey matter just under the dorsal horn. The cells were similar in shape and size to those found in the cat.

76 RELATIONSHIP BETWEEN BARORECEPTOR SENSITIVITY AND CLASSICAL CONDITIONED ARTERIAL PRESSURE RESPONSES IN CATS. Marc A. Nathan, Walter H. Severini*, Lewis W. Tucker*, and Donald J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021.

A common observation is that the magnitude of conditioned arterial pressure (AP) responses is highly idiosyncratic. We recently found enhanced conditioned pressure responses after abolishment of baroreceptor reflexes produced by lesions of the nucleus tractus solitarii (NTS) (Circulation II:144, 1976), indirectly suggesting that the idiosyncrasy of conditioned AP responses may relate to differences in baroreceptor sensitivity. To test this hypothesis, we examined the correlation between baroreceptor sensitivity and the magnitude of conditioned pressure AP responses. Since lability of AP is also increased in cats deprived of baroreceptor function (Circ Res 40:72-81, 1977), the relationship of lability to baroreceptor sensitivity was also examined. Lesions were placed in the NTS in 5 of 11 cats tested. Baroreceptor sensitivity was assessed by calculating the slope of the curve relating the rise in AP, elevated by norepinephrine, to the reflexively induced increase of cardiac interbeat interval. Lability was measured by the standard deviation of a frequency histogram distribution of AP recorded when the cats were not being conditioned. The cats were classically conditioned by presenting two tones of different frequency for 10-60 seconds duration. One tone was immediately followed by delivery of electrical shock and the other tone was never followed by shock. There was a significant decrease in baroreceptor sensitivity in the cats with NTS lesions ($p<.05$). However, within the group the effect of the lesions was variable. When all cats were grouped, correlations could be computed over a wider range of baroreceptor sensitivities than would be normally possible. We found a significant inverse correlation between baroreceptor sensitivity and the magnitude of the conditioned AP response ($p<.05$). A significant inverse correlation was found between baroreceptor sensitivity and the lability of AP ($p<.05$), and lability was positively related to the size of the conditioned AP response ($p<.01$). The high correlation of baroreceptor sensitivity and the magnitude of the conditioned AP response suggests that baroreceptor sensitivity may be an important factor governing the size of conditioned AP pressure responses. The correlation of lability to the conditioned AP response is probably a reflection of the close correlation between lability and baroreceptor sensitivity.

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77 EDINGER-WESTPHAL NUCLEUS: PROJECTIONS TO MEDULLA AND SPINAL CORD. C.B. Saper*, N.D. Yamodis*, and A.D. Loewy (Spon: H. Burton). Depts. Anat. & Neurobiol. and Neurosurg., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Recently we reported that neurons in the Edinger-Westphal (EW) nucleus may be retrogradely labeled with horseradish peroxidase (HRP) after injections in the medulla or spinal cord (Anat. Rec., 187: 640). We now present the results of an autoradiographic study tracing these efferent projections from the EW nucleus. Small injections of a mixture of tritiated amino acids were made in the region of the EW nucleus and after 5 to 7 days survival the brains and spinal cords were processed using the autoradiographic method. Combined with the results of the previous HRP experiments, these new data have allowed us to trace the distribution of some of these fibers and their terminal fields in the medulla and spinal cord. It appears that the main region of termination in the caudal medulla is in a well defined zone between the gracile and cuneate nuclei, which has been previously shown to project to laminae I, IV and V of the spinal cord by way of the lateralmost part of the dorsal columns and the dorsolateral funiculus of the spinal cord, adjacent to the dorsal horn (Burton and Loewy, '77). The main pathways from the EW nucleus to the spinal cord include (i) some fibers described above in the DCN which continue caudally in the ventralmost part and medial septum of the dorsal column and (ii) other fibers which continue caudally along the ventrolateral aspect of the spinal trigeminal nucleus in the zone of the subtrigeminal nucleus and then through the region between the dorsal horn and lateral cervical nucleus to curve around the lateral surface of the dorsal horn. These EW fibers appear to terminate primarily in laminae I and V at least as far caudally as the lower thoracic spinal cord. These experiments indicate that the traditional view of the EW nucleus as merely a parasympathetic preganglionic nucleus should be seriously questioned.

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78 CRYOGENIC BLOCKADE IN THE THALAMIC GATING SYSTEM PREVENTS VENTRICULAR FIBRILLATION IN THE ISCHEMIC MYOCARDIUM OF THE CONSCIOUS PIG. J. E. Skinner and J. C. Reed*. Physiology Dept., Baylor College of Medicine, Houston, TX 77030.

Previous work has shown that the psychologic stress of unfamiliar laboratory surroundings is necessary to initiate ventricular fibrillation (VF) in the conscious pig following left anterior descending coronary artery (LADCA) occlusion (Skinner, et al., Circulation, 51:656-667, 1975). Recent evidence has shown that a tone reinforced by cutaneous shock will evoke slow potentials in the frontal cortex, thalamic reticular nucleus and mesencephalic reticular formation of the conscious cat (Skinner and Yingling, Electroenceph. Clin. Neurophysiol., 40:288-296, 1976). Cryogenic blockade in the pathway interconnecting the frontal cortex and thalamus will abolish these evoked responses to the psychologic stress (tone), but not to the physical stress (shock). The conditioned cardiac response to the tone is also abolished by this cryogenic intervention. Electrophysiological evidence indicates that the frontocortical and mesencephalic projections to the thalamic reticular nucleus, a structure which is itself inhibitory on the thalamic relay nuclei, together regulate the thalamic input to cerebral cortex (Skinner and Yingling, Prog. Clin. Neurophysiol., 1:30-69, 1977). The frontocortical-thalamic reticular system appears to mediate the selective gating associated with "attention" whereas the mesencephalic reticular-thalamic reticular system regulates the more generalized gating associated with "orienting reactions." In conclusion, a single intervention in the frontocortical-thalamic gating system will prevent the selective input to the cortex of a meaningful sensory stimulus and the consequent cardiac response to it. Our hypothesis for the present study, derived from the above neurophysiological evidence, is that intervention in the frontocortical-thalamic reticular pathway will prevent the onset of VF in the ischemic myocardium of the psychologically stressed animal. We found in the chronic pig preparation, stressed by being unadapted to the laboratory, that ventricular fibrillation will occur within 14 min following LADCA occlusion, but if the neural connections between the frontal cortex and thalamic reticular nuclei are blocked cryogenically, then the VF will be postponed or even prevented. Cryogenic blockade in control structures near the frontocortical-thalamic reticular pathway produced no effect on VF latency.

79 EFFECTS OF BICUCULLINE AND THIOSEMICARBAZIDE ON INHIBITION OF REFLEX AND SPONTANEOUS SYMPATHETIC NERVE POTENTIALS IN THE CAT. D. G. Taylor, K. A. Arbour* and M. J. Antonaccio*. Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, N.J. 07901.

A study was made in the α-chloralose-anesthetized cat of the effects of the γ-aminobutyric acid (GABA) receptor antagonist, bicuculline (BIC) and the GABA synthesis inhibitor, thiosemicarbazide (THIO) on the inhibition of spontaneously occurring and somatosympathetic reflex (SSR) induced potentials elicited in the splanchnic and renal nerves. SSR potentials, traveling through medullary pathways, were evoked by stimulation of afferent fibers in a sciatic nerve. Inhibition of spontaneous and SSR reponses was evaluated either during high-frequency (50 Hz) stimulation of the medial medullary depressor region (MDR) or during baroreceptor reflex activation resulting from the hypertensive response to norepinephrine (1-6 μg/kg, iv). BIC (0.1 and 0.3 mg/kg, iv) and THIO (100 mg/kg, iv) produced near total blockade of the MDR-induced inhibition of the SSR. At this time BIC and THIO did not alter baroreceptor-induced inhibition of SSR responses. Blockade of the MDR-induced inhibition commenced 2-5 mins. after injection of BIC and persisted for 30-60 mins. Effects of THIO were maximal 120 mins. after administration. Following BIC or THIO the spontaneous discharges changed from typical cardiac-locked components to sharply spiked activity of larger amplitude exhibiting no apparent relationship to the cardiac cycle. Baroreceptor reflex and MDR activation either completely inhibited these spikes or decreased the spike amplitude and/or frequency of occurrence. The results demonstrate that (1) MDR-induced inhibition of SSR potentials is markedly antagonized by two agents known to interfere with GABAergic transmission, while baroreceptor reflex effects are not changed and (2) spontaneously occurring discharges are altered by GABA antagonism; however, MDR- and baroreceptor-induced inhibition of these spikes remained functional.

80 CALCIUM DEPENDENT POTENTIALS IN THE RAT SUPERIOR CERVICAL GANGLION. Paul Yarowsky and Donald A. McAfee. Dept. Physiology and Biophysics, Univ. of Miami Sch. of Med., Miami, Fl. 33152.

Recent studies, especially in invertebrate nervous systems, have established that action potentials can trigger changes in Ca^{++} conductances which in turn alters membrane potential directly or indirectly by triggering conductance changes to other ions. Few observations have been made in mammalian nervous systems identifying similar phenomena; our studies contribute to the idea that Ca^{++} plays a direct role in control of membrane potential. Postganglionic neurons were impaled under direct visualization with KAc and KCl electrodes (40-80 Megohms). The 150 neurons in this study had an average spike height of 74mV, input impedance of 48 Megohms, time constant of 4.1 msec, and a resting membrane potential of -61mV. Following an action potential elicited synaptically or directly, an afterhyperpolarization (AHP) occured with an average peak amplitude of 8mV, total duration of 300 msec, and a 30% decrease in input impedance. The reversal potential of the AHP was -85mV. Following one second of tetanic synaptic stimulation (30Hz) the duration of the AHP was greatly increased (avg. 1700 msec) with little change in amplitude. The tetanic AHP showed the same reversal potential and resistance change as the single synaptic or direct AHP. The magnitude and duration of the AHP varied directly with the external $[Ca^{++}]$ under conditions where resting membrane potential and input impedance remained unchanged. Several divalent cations Ba^{++}, Co^{++}, & Mn^{++} (1-5mM) and D-600 (20μm) added to the normal Locke greatly reduced the amplitude and duration of the AHP without complete blockade. Tetraethylammonium (5mM) and 4-aminopyridine (3mM) increased the duration of the action potential but had little effect on the AHP. Theophylline (2.5mM) greatly augments the duration of the AHP. Ro20-1724, a non-methylxanthine phosphodiesterase inhibitor had no effect on the AHP. Prostaglandin E_1 (100ηM) depressed both the amplitude and duration of the AHP.

Depolarizing pulses in the presence of TTX elicited a regenerative depolarizing potential as well as an AHP. The amplitudes of both potentials were directly proportional to external$[Ca^{++}]$. The regenerative potential was most prominent when TEA was also present, and had a threshold of -40mV. The AHP occured with a decrease in input impedance and had a reversal potential of -87mV. Both potentials in TTX were blocked by Co^{++}, but only the AHP was blocked by Ba^{++}. The magnitude of the AHP was proportional to the strength and duration of the depolarizing pulses. It appeared that the regenerative potential but not the AHP inactivates. We support the hypothesis that post-ganglionic neurons have a potential sensitive Ca^{++} conductance and a K^{+} conductance which is mediated by Ca^{++}. Supported by USPH grants NS 7044, NS 11552, & NS 05820.

AXONAL TRANSPORT

81 AXONAL TRANSPORT OF CYTOSKELETAL PROTEINS TO THE PRESYNAPTIC TERMINALS IN THE GUINEA PIG VISUAL SYSTEM. Mark M. Black* and Raymond J. Lasek (SPON: Marcus Singer). Dept. Anat., Case Western Reserve Univ., Cleveland, Ohio 44106.

The axonal cytoskeleton, which includes microtubules, neurofilaments and microfilaments, is transported slowly from the cell body towards the axon terminals. The movement of the cytoskeleton and its turnover in the presynaptic terminals has been studied in terms of the individual cytoskeletal proteins. ^{3}H-lysine was injected into the eyes of adult guinea pigs and the animals were sacrificed 6 to 77 days later. The labeled proteins in the superior colliculus (sup. coll.) and in consecutive 3mm segments of the optic nerve and tract were resolved by SDS-polyacrylamide gel electrophoresis.

The results show that the neurofilament proteins (NFP) and the microtubule protein (tubulin) are transported in a slow component (SCa) at 0.5mm/day. The microfilament protein (actin) and its associated proteins are transported in a separate component (SCb) at 1-2mm/day. The bulk of SCb enters the sup. coll. between days 11 and 25 post injection. During this period SCb proteins accumulate in the sup. coll., (apparently within the axon terminals). These proteins then remain there for weeks, and significant amounts are found even 77 days post injection (Fig.). In contrast, SCa proteins (which begin entering the sup. coll. on days 40-45 post injection) do not accumulate (Fig.) but turnover within a few days of entering. Since actin and its associated SCb proteins reside in the axon terminals for weeks it is likely that these cytoskeletal proteins are important constituents of the presynaptic terminal and are thus involved in transmitter release.

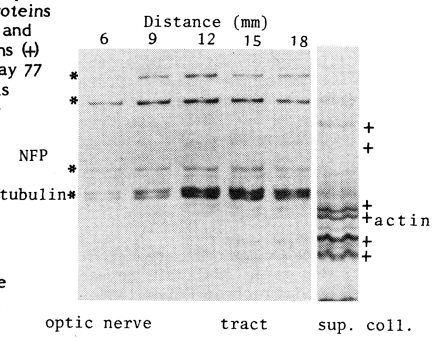

Figure. This fluorograph shows labeled SCa proteins (*) in the optic nerve and tract and SCb proteins (+) in the sup. coll. at day 77 post injection. At this time one third to one half of the total labeled SCa has entered the sup. coll., but very little remains. In constrast, a substantial amount of the labeled SCb, which had entered the sup. coll. earlier than the SCa, is still there at 77 days.

82 ANTEROGRADE AXONAL TRANSPORT OF PEROXIDASE: HOW SIGNIFICANT? R. D. Broadwell and M. W. Brightman*. NIH, Bethesda, MD 20014.

Using the neurosecretory system of the mouse hypothalamus as an example, the anterograde transport of exogenous horseradish peroxidase (HRP) in undamaged neurons has been investigated by electron microscopy under normal and experimental conditions. Cerebral ventriculo-cisternal perfusion of 10 µl of a 30% HRP mixture resulted in a rapid diffusion of the protein throughout the brain. The posterior pituitary, however, consistently appeared devoid of reaction product extracellularly at all survival times ranging from 5 mins to 48 hrs. By 12-24 hrs, perikarya of the neurosecretory neurons in the supraoptic and paraventricular nuclei were heavily labeled with HRP, due to direct pinocytotic activity of the cell bodies and dendrites and to retrograde transport in axon collaterals situated outside the posterior pituitary. Between 6 and 48 hrs post-injection, very few neurosecretory axons and terminals contained HRP-labeled organelles. These organelles consisted of tubular profiles of smooth endoplasmic reticulum (ER) and dense bodies. The latter were, like neurosecretory granules, about 2000 A-wide. The paucity of labeled organelles transported anterogradely in the pituitary stalk axons stands in sharp contrast to the large number of HRP-labeled organelles within these same axons labeled retrogradely following vascular injection of the protein.

In an attempt to increase the anterograde flow of peroxidase in the neurosecretory system, 6 mice were dehydrated by having them drink a 2% NaCl solution for 5 days prior to ventricular perfusion of HRP. The animals were then fixed 12-24 hrs post-injection. In each case, the anterograde transport of HRP-labeled organelles in the pituitary stalk axons was markedly increased. The majority of axons in the pituitary stalk and many terminals in the posterior pituitary contained numerous HRP-labeled smooth ER and 2000 A-wide dense bodies. The large number of labeled organelles in the pituitary stalk axons of NaCl-treated animals rivaled that found in retrograde transport in stalk axons of vascular injected mice. The labeled 2000 A-wide dense bodies are probably lysosomes and not neurosecretory granules. The number of these labeled organelles did not diminish and no reaction product was seen extracellularly in the posterior pituitary in response to hemorrhage, a potent stimulus for hormone release from the posterior pituitary.

These results suggest that, normally, anterograde transport of HRP in undamaged neurons is negligible. The bulk of exogenous peroxidase that enters the perikaryon remains there for eventual degradation. In instances of severe physiological stress, anterograde transport of HRP may be increased. Such an increase could reflect an added need for degradative organelles in the more distal compartments of the neuron.

83 MORPHOLOGIC ANALYSIS OF UPTAKE AND RETROGRADE TRANSPORT OF PEROXIDASES BY AXONS. Ann H. Bunt and Richard H. Haschke. Depts. of Ophthalmol. and Anesthesiol., Univ. of Wash., Seattle, WA 98195.

The retrograde axonal transport of horseradish peroxidase (HRP) in the rat visual system is highly specific. Thus, of a number of proteins examined, only the basic isoenzymes "C" and "B" but not a mixture of the acidic isoenzymes "A" of HRP are taken up by axons of retinal ganglion and dorsal lateral geniculate cells and transported retrogradely to their somata. The molecular features of the enzymes which might underlie this specificity have been examined using biochemical and morphologic techniques. Substitution of the positively charged amino groups on isoenzyme C with negative carboxyl groups by succinylation changed the isoelectric point (pI) from 8.1 to 4.0 without affecting the sugars or enzyme activity and caused noticeable depression of retrograde transport. However, a basic pI is not the sole requisite for retrograde transport since a peroxidase isolated from turnip (courtesy of Dr. G. Mazza) possessing a similar carbohydrate composition and molecular weight, with a pI of 11.6, and also cationized A isoenzymes (approximately neutral pI) were not transported. The turnip peroxidase did cause striking filling of neuronal somata and processes for long distances away from the injection sites. Peroxidase obtained recently from local horseradish roots have yielded two distinct acidic isoenzymes. The more basic of the two (A_b; pI = 4.9) shows no detectable retrograde transport, while the more acidic isoenzyme (A_a; pI = 4.2) shows weak to medium transport. Lactoperoxidase (pI = 8.0) and one batch of Sigma HRP VI (65C-9530-1) also are not transported. Removal of calcium from isoenzyme C followed by carboxyl group modification caused significant depression of enzyme activity (2400 to 300 units/mg) but did not affect retrograde transport, indicating that very low levels of enzyme activity are detectable by our light microscopic methods. E.M. of superior colliculus and visual cortex around the injection sites after various time intervals revealed the uptake of HRP isoenzymes C, A_b, and A_a, lactoperoxidase and turnip peroxidase into synaptic and coated vesicles and fine tubules of the synapses. Only HRP isoenzymes C and A_a were found in multivesicular bodies in synapses and axons, or within membrane-bounded granules in neuronal somata. The Sigma VI (65C-9530-1) did not enter any of these membraneous compartments. Thus, while many proteins enter the vesicular organelles associated with neurotransmitter recycling in synapses, only the HRP isoenzymes C and A_a enter the vesicles responsible for retrograde transport in axons. The roles played by the individual sugar moities and protein conformation in this specificity are currently under investigation. (Supported by NIH Grants EY 01756 and 01311 and GM 15991).

84 SELECTIVITY AND KINETICS OF PEROXIDASE UPTAKE BY SYNAPTOSOMES AND NEUROBLASTOMA CELLS. Kwan Y. Chan*, Ann H. Bunt, and Richard H. Haschke (SPON: W.K. Dong). Dept. of Anesthesiology, and Ophthalmology, University of Washington, Seattle, WA 98195.

To investigate further the selectivity of uptake and retrograde axonal transport of horseradish peroxidase (HRP) observed in the visual system, the uptake of various peroxidases into rat brain synaptosomes and differentiating mouse neuroblastoma N18 cells were studied. When synaptosomes were incubated with HRP isoenzyme C (HRP-C) (2 mg/ml) at 30°C for 15 to 30 min, the cytochemical reaction product of the peroxidase was observed on the surface of synaptosomes and inside synaptic vesicles, coated vesicles, multivesicular bodies, and vacuoles. When differentiating N18 cells were incubated with the same concentration of HRP-C at 37°C, peroxidase was found first on the cell surface and appeared in coated pits and endocytic vesicles (0.1 µ) within the first few minutes. By 5 min, peroxidase was found in vacuoles (0.3 to 0.5 µ), and by 15 to 20 min, in various homogeneous and heterogeneous dense bodies of similar sizes. The presence of peroxidase inside these structures was readily demonstrated in both neurites and soma of the cell. HRP isoenzyme A, succinylated HRP-C, Sigma HRP type VI (lot 65C-9530-1, which is not taken up nor transported retrogradely by the visual system), and to a lesser extent, periodate-borohydride-treated HRP-C (pb-HRP-C), were also taken up into similar structures of synaptosomes and N18 cells. However, turnip peroxidase, lacto-peroxidase and cationized ferritin were generally not incorporated into the various membraneous structures of synaptosomes and N18 cells under similar conditions of incubation. Thus, although synaptosomes took up the different HRP's, presumably through synaptic vesicle membrane recycling, while N18 cells ingested HRP's through endocytic-lysosomal activity, these two systems expressed identical membrane selectivity of peroxidase uptake in vitro. This selectivity, however, deviates from the selectivity of peroxidase uptake expressed by several axon types of the visual system in vivo with respect to Sigma HRP type VI (lot 65C-9530-1). We conclude that neuronal membranes in an in vitro environment may be altered so that subtle changes in HRP molecules may not be operant in influencing uptake, but that selectivity against uptake of proteins with greater molecular differences is still maintained.

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85 MAYTANSINE INDUCED BLOCK OF AXOPLASMIC TRANSPORT AND MICROTUBULE LOSS. B. Ghetti and S. Ochs. Depts. of Pathology and Physiology, Indiana University School of Medicine, Indianapolis, Indiana 46202.

As part of our interest in the relation of axoplasmic transport to microtubules in a proposed transport filament model, the action of maytansine (MTS), an ansa macrolide which binds to tubulin, was studied. The action of MTS was compared with other agents, also under study, e.g. colchicine, vinca alkaloids, batrachotoxin (BTX). An investigation of the effect of MTS on both axoplasmic transport and on the axonal microtubules was carried out. Because the perineurial sheath is an effective permeability barrier, the desheathed cat peroneal nerve preparation was used (Ochs, Worth and Chan, Abst. Soc. Neurosci., this meeting, 1977). The L7 ganglia were injected with ^{3}H-leucine and 2 hours of downflow allowed before the nerves were removed, the peroneal nerve desheathed over a length from 40 to 130 mm from the ganglion and these nerves placed in flasks containing 35-70 μM MTS in lactated Ringer for 2 to 4 hours of in vitro downflow. After this period of time a 15 mm portion of nerve was removed from the end, fixed in 5% glutaraldehyde and processed for electron microscopy. The remainder of the nerve was used to determine the outflow of the labelled activity and thus to characterize the effect of MTS on axoplasmic transport.

A marked reduction of transport was found at the shorter times with 70 μM MTS while nerves exposed to 35 μM showed a smaller decrease of transport. On a comparative basis, MTS appeared to be somewhat less potent than vinblastine and vindesine in blocking axoplasmic transport and much less potent than BTX. Ultrastructural examination of myelinated axons showed a substantial decrease in the number of microtubules in the nerve fibers exposed to 70 μM of MTS. In the unmyelinated fibers, where normally microtubules are relatively more prominent, their disappearance was even more dramatic. An occasional microtubule could be seen in fibers on careful searching; however, the majority of microtubules have disappeared. Microtubules also disappeared from the Schwann cells. Within the nerve fibers, especially the unmyelinated ones, microfilaments appeared to be intermingled with a greatly increased amount of amorphous floccular material. Only a minor loss of microtubules was seen in nerves exposed to 35 μM MTS at shorter times with more loss at the longer times. To show the suggested relation of transport block to decrease of microtubules at concentrations in the range of 35-70 μM of MTS as a function of time, microtubular counts are being correlated with the degree to which the transport is diminished. Supported in part by NIH grants PHS SO1 RR 5371 and PHS RO1 NS 8706-08; and NSF grant BNS 75-03868-A03.

86 A NEW SPECIFIC, SENSITIVE AND NONCARCINOGENIC REAGENT FOR THE DEMONSTRATION OF HORSERADISH PEROXIDASE(HRP). Jacob S. Hanker, Peggy E. Yates*, Carol B. Metz*, Keith A. Carson, Alan Light and Aldo Rustioni, Dent. Res. Ctr., Neurobiol. Progr., Depts. of Anat. and Physiol. UNC, Chapel Hill, N.C. 27514

In the course of studies of the formation of artificial melanin-like polymers it was found that the peroxidation of p-phenylenediamine (PPD) was greatly accelerated by the presence of pyrocatechol (PC). The copolymer formed as a result of this oxidative coupling reaction was insoluble, conformed well to biological ultrastructure, and gave a bluish color more intense than that obtained with 3,3-diaminobenzidine (DAB). It was also osmiophilic. Peroxidation of the individual components resulted in unsatisfactory staining.

The reagent mixture (1:2 proportion of PPD to PC)[1] outperformed DAB as an indicator for the demonstration of HRP in mammalian cells including neurons and supporting cells. It is more sensitive to and specific for plant hydroperoxidases or oxygen transferases than the corresponding mammalian enzymes. Thus there is less interference due to erythrocytic hemoglobin or erythrocytic or endogenous cytoplasmic catalases and peroxidases than when DAB is employed. Its superiority for demonstrating HRP in phagosomes of many cell types including Schwann cells was shown. In proximal convoluted tubular epithelial cells, histiocytes, macrophages, osteoclasts and Schwann cells, the HRP sequestered by the limiting membranes of phagocytic vacuoles was much more clearly demonstrated with PPD-PC[1] than with DAB. In retrogradely labelled neurons of the ventrolateral nucleus of the thalamus, dorsal column nuclei and spinal cord, the reaction product was significantly more intense with 15 min. PPD-PC[1] incubation than with 30 min. DAB incubation after 30% HRP injections in motor cortex and n. ventralis posterolateralis of thalamus of cats and rats.

Likewise in single primary afferent fibers labelled by anterograde transport of intracellularly injected HRP, the reaction product produced by PPD-PC[1] was more readily visible than tissue reacted with either DAB or tissue pre-treated with $CoCl_2$ followed by reaction with DAB (J.C. Adams, Neuroscience 2, 141-145, 1976).

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[1]Although these compounds are noncarcinogenic, they are skin irritants and it is advisable to use the stable premixed reagent which is available from Polysciences, Inc., Paul Valley Industrial Park, Warrington, Pa., 18976 USA.

87 BIOCHEMICAL REQUISITES OF HORSERADISH PEROXIDASE ISOENZYMES RELATED TO RETROGRADE AXONAL TRANSPORT. Richard H. Haschke and Ann H. Bunt. Departments of Anesthesiology and Ophthalmology, University of Washington, Seattle, WA 98195

The uptake and retrograde transport of exogenous proteins by axons is a selective process which may involve specific biochemical interactions between the neuronal cell membrane and the protein being taken up. The aim of this biochemical study is to determine what biochemical parameters regulate the uptake and retrograde transport of horseradish peroxidase (HRP). By studying various isoenzymes of HRP, chemically modified forms of HRP, and other similar peroxidase enzymes, we are attempting to better understand this selective recognition process. Initially a clear correlation seemed to exist between the isoelectric point (pI) and the retrograde transport of various proteins. However, more recent experiments have demonstrated that indeed one acidic isoenzyme of HRP is also transported retrogradely. The basic and acidic isoenzymes all contain essentially identical sugar moieties consisting of N-acetylglucosamine, mannose, fucose, xylose, and arabinose. Both transporting and non-transporting isoenzymes of HRP have molecular weights of ca. 44,000 and exist in the monomeric form. Amino acid analyses performed on the peroxidase isoenzymes indicate that the basic and acidic isoenzymes are similar in composition and most likely differ in the isoelectric point because of a large difference in the content of amide groups. Titrations under denaturing and non-denaturing conditions have indicated that the free carboxyl content of the two proteins differs markedly and show structural differences as based on the reactivity of the carboxyl groups. HRP has also been shown to contain two moles of bound Ca^{++} per mole of enzyme. Removal of Ca^{++} from HRP results in 50% decrease in enzymatic activity with the C isoenzyme and 90% decrease in activity of isoenzyme A. Addition of Ca^{++} free HRP results in complete reconstitution and return of enzymatic activity with the C isoenzyme. In contrast, the A isoenzyme does not recombine with Ca^{++} and does not regain enzymatic acitvity. Exchange of Ca^{++} containing HRP isoenzymes with free Ca^{++} results in rapid and complete exchange with isoenzyme C, but essentially no detectable exchange with isoenzyme A. These differences between isoenzymes C and A with respect to Ca^{++} reconstitution and exchange suggest definite structural differences between these proteins. We propose that the biochemical basis of selective recognition of certain proteins by axons may not depend simply on the presence of carbohydrate and a basic pI, but may be related to a specific conformation which exposes certain groups to the cell membrane in the proper configuration for the recognition and uptake into retrogradely transported vesicles to occur. (NIH grants EYO 1756 and 1311, and GM 15991)

88 FAST AXONAL TRANSPORT OF GLYCOPROTEINS: COMPLETE INHIBITION BY COBALT IONS. Pierre-André Lavoie* and Richard Hammerschlag. Div. of Neurosciences, City of Hope Nat. Med. Ctr., Duarte,CA 91010.

Glycoproteins labelled with [^{3}H]fucose or [^{3}H]glucosamine undergo axonal transport at the same fast rate as proteins marked with radioactive amino acids. Among a variety of preparations, this was shown in vitro in the bullfrog spinal-sciatic nerve complex (Edström & Mattsson, J.Neurochem.19:1717, 1972), and confirmed in a similar preparation in the present study. With [^{3}H]fucose and [^{3}H]glucosamine as precursors, accumulation of TCA-insoluble radioactivity proximal to a ligature 20 mm from the ganglion reached a maximum in 18 h. Maximal accumulation of protein labelled with [^{3}H]leucine was also observed at 18 h. The maximal accumulation of [^{3}H]glycoprotein at the ligature differed, however, from that seen for total [^{3}H]protein; a peak ratio (net accumulation at ligature: plateau level) of 6 was observed with carbohydrate precursors compared to 9 with [^{3}H]leucine. Given that the transport rate is similar for [^{3}H]leucine- or [^{3}H]carbohydrate-labelled proteins, this difference may suggest that relative to the total pool of protein undergoing axonal transport, glycoproteins are preferentially removed from the transport system in non-synaptic regions of the axon. The effect of cobalt on glycoprotein transport was then examined as part of our continuing study of the calcium requirement for fast axonal transport of protein (Hammerschlag, Chiu, & David, Brain Res.114:353,1976). While the amount of total [^{3}H]protein transport was blocked by 80-90% in an incubation medium containing 1.8 mM $CoCl_2$, the inhibition of [^{3}H]glycoprotein transport was essentially 100% under similar conditions. This blockage of glycoprotein transport was seen by the absence of accumulated radioactivity proximal to a ligature on the 8th spinal nerve, as well as by the lack of observable radioactivity in those segments of non-ligated 9th spinal and sciatic nerve corresponding to the plateau and crest regions of radioactivity in the contralateral control nerve. Thus, the residual protein material that is transported in the presence of cobalt does not contain fucose or glucosamine and may not be glycoprotein in nature. The effect of cobalt on glycoprotein transport seems not to be a result of inhibition of protein glycosylation as judged by the total incorporation of [^{3}H]fucose and [^{3}H]glucosamine into TCA-insoluble materials. In previous experiments, cycloheximide was utilized to show that cobalt was also not affecting transport by blocking protein synthesis. In light of the proposal of a calcium-dependent,cobalt-sensitive step in the initiation of fast axonal transport of protein, the present findings imply that this step occurs following protein glycosylation and prior to the onset of translocation of proteins via the transport system.
(Supported by MRC of Canada Fellowship (P.-A.L.), and by NSF Grant BNS75-17640.)

89 AXONAL TRANSPORT OF [^{3}H] PROTEINS IN RAT BRAIN NORADRENERGIC NEURONS. Barry E. Levin* (SPON: S.D. Cook). Department of Neurosciences, Veterans Administration Hospital, East Orange, NJ 07019 and New Jersey Medical School, Newark, NJ 07103.

Axoplasmic transport of proteins was studied in the noradrenergic pathway from the locus coeruleus (LC) to the hypothalamus of the rat using [^{3}H] leucine injected stereotaxically into the LC. [^{3}H]protein synthesized from [^{3}H]leucine was transported in four waves through the hypothalamus at rates of 96, 48, 26 and 1-2 mm/d (waves I, II, III and IV respectively). Specificity of transport within catecholamine neurons was demonstrated by 6-hydroxydopamine lesions of the LC-hypothalamic pathway which blocked transport of all four waves, while a fifth wave of uncertain origin, was not blocked. Such lesions cause a blockade of orthograde dopamine-B - hydroxylase transport as demonstrated by immunofluorescent histochemistry and a 30% decrease in enzyme activity in the ipsilateral frontal cortex without change in hypothalamic levels. Waves I and II correspond in rate to those for fucosyl glycoproteins (Brain Research, in press) and wave II, to the rate of norepinephrine transport in this model (Brain Research, 120: 303, 1977). The various waves have different properties by ion exchange chromatography suggesting the heterogeneity of transported proteins in each wave.

90 DEPENDENCE OF AXOPLASMIC TRANSPORT ON CALCIUM SHOWN IN THE DESHEATHED PERONEAL NERVE. Ochs, S., Worth, R.* and Chan, S. Y.* Department of Physiology and Medical Biophysics Program, Indiana University School of Medicine, Indianapolis, Indiana 46202.

On the basis of the transport filament hypothesis for fast axoplasmic transport, we would expect Ca and perhaps Mg to play an important role. Prior studies showed an apparent lack of effect of Ca-free media on axoplasmic transport in vitro. The perineurial sheath acts as an effective permeability barrier for a number of materials and resists changes of the ionic concentration in the extracellular fluid held within it. A desheathed nerve preparation was developed to assess the effect of different ionic compositions in the medium on axoplasmic transport. The peroneal branch of the sciatic nerve was desheathed from about 35 mm down to about 130 mm from the L7 dorsal root ganglion. This permitted a relatively long length of desheathed nerve to be exposed to a given medium for over 4 hrs of downflow in vitro. The L7 dorsal root ganglia were injected with ^{3}H-leucine and 2 hrs of downflow in vivo allowed before the sciatic nerves were removed. The peroneal branch of the sciatic nerve was desheathed and the tibial branch left intact as a control. The nerves were placed in flasks containing 20 ml of medium, either lactated Ringer, or isotonic NaCl or sucrose with or without Ca added. The solutions were vigorously bubbled with 95% O_2 + 5% CO_2 at 38°C. With Ringer or with 5 mM $CaCl_2$ present, transport in the desheathed branch compared favorably with that seen in the sheathed branch. The crest was not as prominent but otherwise the usual rate of transport of 410 mm/day was maintained. When Ca was removed from the medium, transport in the desheathed peroneal branch fell off and failed completely within approximately 2 hrs. Mg was not able to substitute for Ca in supporting transport. We consider that the removal of Ca from the medium causes the level of ionized Ca in the fibers to fall below a critical level required to maintain transport. A Ca-binding protein, sequestration of Ca in mitochondria and possibly also in the endoplasmic reticulum, Na-Ca exchange carrier and a Ca-pump in the membrane are all possible mechanisms acting to maintain free Ca at the level required for axoplasmic transport. With higher concentrations of Ca present in the in vitro medium, the rate of axoplasmic transport was gradually decreased. With isotonic Ca, block occurred within 1-2 hrs. An augmentation of Ca in the fibers could interfere with axoplasmic transport by blocking the same mechanism affected by removal of Ca, or alternatively, by some other action on the microtubules. Supported in part by the NIH grant PHS RO1 NS 8706-08 and the NSF grant BNS 75-03868-A03.

91 AXONAL TRANSPORT OF CHOLESTEROL AFTER INTRAOCULAR INJECTION OF MEVALONIC ACID. John A.P. Rostas* and Peter L. Jeffrey*, (Spon: J.C. Blosser) Dept. of Psychobiology, Univ. of California, Irvine, Ca. 92717 and Dept. of Neurology, Baylor College of Medicine, Houston, Tx. 77030.

After the injection of labeled mevalonic acid, a metabolic precursor of cholesterol, into the eye of young chickens, both the fast and the slow phase of axonaly transported radioactivity can be detected at the contralateral optic tectum. Seventy five percent and ninety two percent of the radioactivity in the fast and slow phases respectively, was recovered in the free cholesterol. After the injection of labeled cholesterol into the eye, radioactive cholesterol was axonaly transported only at the slow rate: no fast rate of cholesterol transport could be detected (Rostas et.al. (1975) J. Neurochem. 24:295-302). Therefore, in the chick optic system, cholesterol is axonaly transported in two phases, which appear to take their cholesterol from different cellular pools. The fast phase appears to be specific for newly synthesized cholesterol whereas the preformed, endogenous cholesterol can readily exchange with the transport pool for the slow phase. Because these two pools for transport phases can be selectively labeled, the retina and optic nerve provide a unique model system in which the metabolic turnover, intracellular compartmentalization, and intracellular transport of cholesterol can be studied.

92 RAPID PARTICLE TRANSPORT IN AXONS FROM DECAPOD CRUSTACEANS. Richard S. Smith. Dept. Surgery, Univ. Alberta, Edmonton, Canada.

A search has been made among the invertebrates for large diameter, non-myelinated axons in which the rapid axoplasmic transport of optically detectable particles takes place. In general, the peripheral nervous systems of the decapod crustacea provide suitable preparations.

Single "giant" motor axons were dissected from nerve trunks of the walking legs of several genera of crabs, two species of lobsters and the crayfish Pacifastacus. The axons were viewed in saline filled chambers by darkfield and Nomarski microscopy. All nerve fibers showed a sub-axolemmal system of axially oriented filaments which individually were up to 50 μm in length. These corresponded in position to the circumferential mitochondria and smooth endoplasmic reticulum seen by thin-section electron microscopy. The sub-axolemmal filaments were stationary. The core of the fibers contained long filamentous bodies, rods of a few μm length and sub-micrometer particulate bodies. All classes of bodies detected in the core of the axons showed some form of motion, however, the particulate bodies had the most regular motion and were the most rapidly moving. All nerve fibers contained proximally (somatopetally) moving particles with average velocities of about 1 μm/sec at room temperature. Values for these velocities are given as means (μm/sec) ± SD with the number of determinations in parentheses followed by the temperature in °C: Carcinus meanus, 1.08 ± 0.28 (65), 19.5-20; Homarus vulgaris, 1.01 ± 0.30 (58), 19-20; Pacifastacus trowbridgeii, 1.04 ± 0.39 (92), 21-22. The velocities of proximally moving particles in C. meanus and H. vulgaris had a Q_{10} of 2.3 and 3.0 respectively over the range of 16-27°C. Distally (somatofugally) moving particles were always fewer than proximally moving ones; their number was variable and some axons contained no detectable distally moving particles. Their velocities were less than those of proximally moving particles, these were, with the convention as above: C. meanus, 0.40 ± 0.10 (40), 19.5-20; H. vulgaris, 0.34 ± 0.12 (20), 19-20; P. trowbridgeii, 0.40 ± 0.12 (39), 21-22. The Q_{10} for the velocity of distally moving particles was, in C. meanus, 2.1 and in H. vulgaris, 1.9.

Particle transport in these axons is unique in that material moves faster somatopetally than somatofugally.

(Supported by the Medical Research Council of Canada.)

93 TWO-DIMENSIONAL MAPPING OF PROTEINS IN RAPID AXOPLASMIC TRANSPORT. George C. Stone, David L. Wilson, and Michael E. Hall. Dept. of Physiol. and Biophys., Univ. of Miami, Sch. of Med., Miami, Fl. 33152.

Rapid axoplasmic transport is thought to include proteins primarily involved in nerve terminal maintenance rather than axonal growth. Previous findings have suggested that at least 6 to 24 protein species are present in this type of transport.

Modifications of O'Farrell's (1) procedure for two-dimensional electrophoresis of proteins has allowed high resolution analysis of the protein species rapidly transported in the frog sciatic nerve.

The 7th, 8th, and 9th dorsal root ganglia were selectively labeled with ^{3}H-leucine, ^{14}C-leucine, or ^{35}S-methionine in one compartment of a lucite chamber. Transport of TCA precipitable material was monitored in the spinal roots and sciatic nerve kept in another compartment. Fastest transport rates were 75-90mm/day at 18°C. Ligation of the nerve 30mm distal to the 8th ganglion at the beginning of the experiment resulted in accumulation of label during a 24 hr period. This material was subjected to two-dimensional electrophoresis (pI 4.5-8.5; m.w. 10^4-10^5 daltons) in 3mm nerve segments. Autoradiographs or fluorographs from segments proximal to the ligature yielded a pattern of at least 60 labeled proteins. Neither actin nor tubulin were present among these rapidly transported, labeled proteins. No pattern was observed from segments distal to the ligature, ruling out the possibility of local incorporation by contamination. Blocking protein synthesis with 18μM anisomycin reduced the accumulation of label proximal to the ligature by 98%. Direct labeling of nerve segments produced patterns significantly different from the pattern of transported proteins. A comparison of proximal segment staining with labeled incorporation in the same segment following transport revealed at most 12 common species. It is concluded that at least 48 of the transported protein species are neither synthesized nor found in abundance in nerve segments. These may be destined for nerve terminals.

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(1) O'Farrell (1975) J. Biol. Chem. 250; 4007.

94 SECTIONING OF THE RAT SCIATIC NERVE PRODUCES CHANGE IN SPECIFIC PROTEINS CARRIED BY RAPID AXOPLASMIC TRANSPORT. Richard F. Theiler* and William O. McClure. Dept. of Biochemistry, Univ. of Illinois, Urbana, IL 61801, and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90007.

One sciatic nerve of a rat was exposed and sectioned. At times of 5 - 50 days after section, both the sectioned nerve with its associated dorsal root ganglion (drg), and the contralateral nerve and drg, were excised and transferred to short term in vitro culture. The drg were labelled with radioactive amino acids, after which those proteins committed to transport were detected by their acculation at a ligation placed on the nerve proximal to the cut end. Accumulation of transported proteins was allowed to proceed for 18 hr at 37° in O_2:CO_2 (95:5). Under these conditions, transported radioactivity included only material which moved at rates $\gtrsim$ 50 mm/day. The amount of transported radioactivity was compared in sectioned nerves and their contralateral controls. No statistically significant differences were observed. Similarly, no significant differences were observed in the amount of precursor incorporated into proteins of the two drg. Transported proteins were solubilized, fractionated on slabs of polyacrylamide in sodium dodecyl sulfate, and visualized by radioautography after the gel was impregnated with PPO. Several changes of individual bands occurred as a result of nerve section. One protein (MW 19000 daltons) disappeared from the transported flow following section, while a second (MW 42000 daltons) appeared. Both proteins altered their levels with a time course which followed histologically defined chromatolysis in the animals. In addition to the changes in the sectioned nerve, we also detected changes in the proteins transported by the control (unsectioned) sciatic nerve of an animal in which the other nerve was sectioned. Two proteins which are carried by axoplasmic transport in sciatic nerves of unoperated animals are not detectable in the material transported by control nerves of operated animals. The two proteins disappear for two weeks after section, and reappear at later times. These changes may be related to compensatory use of the limb contralateral to a sectioned nerve. It is possible that the rapidly transported proteins whose levels are altered in sectioned nerves are involved in the control of neuronal regeneration. Supported in part by the National Institutes of Health (NS 09082, 13215), the Alfred P. Sloan Foundation, the Muscular Dystrophy Associations of America, and the Nelson Research and Development Company.

95 POLY-L-ORNITHINE IMPROVES LIGHT MICROSCOPIC VISUALIZATION OF HORSERADISH PEROXIDASE. Michael C. Trachtenberg, and Robert T. Hadley*. Neurology Svc., Boston VA Hospital, and Dept. of Neurology, B.U. Sch. of Med., Boston, Mass. 02130.

Poly-L-ornithine (ORN) mixed with horseradish peroxidase (HRP) increases the amount of HRP uptaken, greatly magnifying signal amplitude and rendering it visible for a longer time. This effect is of major importance for light microscopy where maximal resolution is ~200 nm, while vesicles containing HRP are 20-100 nm in diameter. The production of multivesicular bodies containing adequate numbers of sufficiently labelled vesicles is a time, system and direction dependent process, evident in four distinct problem areas. These are not now adequately solved by the use of different types of HRP or special fixation or reaction procedures which can only better reveal available HRP.

Paired injections of various concentrations of ORN (m.w. 200,000) with 25% HRP and 25% HRP alone were made in frontal agranular and visual cortex of Long Evans rats. After survival times of 2.5 - 100h animals were sacrificed, perfused and processed by several techniques. Four subcortical loci were examined; claustrum, ventral tier complex, lateral geniculate nucleus (LGN) and lateral dorsal nucleus (LD). Four to six times as many retrogradely labelled cells are seen in experimental as compared to control loci at post-injection times of 5-72h. Using ORN-HRP or HRP alone label is first visible and disappears at similar times. Cell counts as a function of time indicate that under test and control conditions only the number of cells and not their distribution differ suggesting an alteration in the amount of HRP transported but not in the transport rate. Anterograde terminals are seen at all times in LGN, LD and medial dorsal nucleus on the experimental side when few or none are visible on the control side.

The increased reliability of visualizing both retrograde and anterograde transport of HRP attributed to ORN allows full utilization of the entire temporal window for labelling: 5-100h vs. 16-48h by standard methods. Consequently ORN can be used advantageously to trace very long as well as short connections simultaneously in large primate brains. Four problems which appear to be ammenable to solution by this procedure are: a) inability to demonstrate by retrograde transport some known connections; b) low reliability of seeing anterograde terminals; c) inadvisability of using very small amounts of HRP to label discrete regions; d) restriction of optimal survival times.

96 PARGYLINE INDUCES CHANGES IN AXONAL TRANSPORT IN MOTOR NEURONS. D.F. Watson, J.A. Donoso and F.E. Samson. Ralph L. Smith Mental Retardation Center, University of Kansas Medical Center.

Pargyline (N-methyl-N-benzyl-2-propynylamine hydrochloride) is a monoamine oxidase inhibitor which produces numerous changes in neural and muscular functions. Because of previous conflicting reports, we have re-examined the possibility that pargyline can induce an apparent increase in the velocity of axonal transport. Male Holtzman rats, 450-550 gm, received 3 daily doses of pargyline (75 mg/kg, i.p.; controls received i.p. sterile water. Eleven of 31 rats died during the pargyline treatment. One day after the last dose, 50 uCi of 3H leucine (61 Ci/mmole) in 2.5λ of Locke's solution was micro-injected bilaterally into the L5-6 ventral horn regions under sodium pentobarbital anesthesia. After 1-4 hr for incorporation and transport, the sciatic nerves and L5 ventral roots were removed, rapidly frozen, fixed in 95% ethanol or 5% trichloracetic acid, and cleaned of connective tissue. Tritium cpm per 2 mm nerve segment were determined by digestion and liquid scintillation counting. The bulk of the transported material in the pargyline-treated rats moved at the control velocity (11.9±3.5 mm/hr and 13.3±2.2 mm/hr, respectively. However, pargyline also induced faster components of transport. In all 10 pargyline-treated nerves, a faster but smaller peak was observed, with a mean velocity of 21.4±5.1 mm/hr; in addition, 6 of 10 nerves showed a small peak at a rate of 39.9±6.8 mm/hr. Preliminary results suggest that pargyline does not cause such changes in dorsal root preparations. The observed changes in axonal transport may be a result of the accumulation of monoamines due to pargyline; however, other modes of action for pargyline cannot be excluded.
(Supported in part by the U.S.P.H.S. grant HD-02528)

BASAL GANGLIA

97 ELECTROPHYSIOLOGICAL STUDIES OF BRANCHING NIGRAL AXONS AND THE MONOSYNAPTIC INHIBITION PRODUCED IN THALAMIC NEURONS BY STIMULATION OF THE SUBSTANTIA NIGRA. M.Anderson, M. Yoshida*, and A. Ueki*. Depts of Rehab. Med. and Physiol. and Biophys., Univ. of Wash., Seattle, and Dept of Neurology, Jichi Medical School, Tochigiken, Japan.

Anatomical studies have shown that the substantia nigra (SN) sends axons to the ventromedial thalamus (VM) and to the superior colliculus (SC). We have studied the collicular and/or thalamic destination of axons of single SN neurons and the synaptic potentials produced in thalamic neurons by SN stimulation.

Cats were anesthetized with alpha chloralose or sodium pentobarbital. In some experiments, recording microelectrodes were inserted into SN during stimulation of ipsilateral VM and SC. Extracellularly-recorded action potentials of negative configuration were identified as antidromic based on constant latency, high frequency-following and collision block criteria. Three groups of cells were activated antidromically, from (1) the thalamus only, (2) SC only, or (3) both thalamus and SC. Threshold stimulus intensities were lowest in the region of VM and in intermediate to deep layers of SC. Mean antidromic latencies were 1.17 msec from SC and 1.66 msec from the thalamus.

In other experiments, microelectrodes were inserted into the thalamus from a lateral angle of 30° and the ipsilateral SN and contralateral brachium conjunctivum (BC) were stimulated. Intracellular records from neurons in VL showed EPSPs evoked at short latencies by BC stimulation, but no response to SN stimulation. However, in VM neurons, SN stimulation evoked an IPSP at short latencies, but there was no response to BC stimulation. The short, constant latency of the nigral evoked IPSP (1.2-2.0 msec in different cells) and its constant configuration made it probable that it was produced monosynaptically. Furthermore, if the IPSP was produced by stimulating SN fibers closer to VM, the latency was reduced markedly, and if the conduction distance was extrapolated to 0mm, the delay was too short for more than one synaptic relay.

Thus, at least some SN neurons appear to send axonal branches to both VM and SC, and axons from nigral neurons inhibit VM neurons that are not directly excited by cerebellar output.

Supported by RSA grant 16 P 56818.

98 RETROGRADE AXONAL TRANSPORT OF HERPES SIMPLEX VIRUS IN BASAL GANGLIA AND RELATED NUCLEI. Il Jin Bak*, Charles H. Markham, Margery L. Cook* and Jack G. Stevens* (SPON: Hilde E. Hirsch). Dept. Neuro., Sch. Med., UCLA, Los Angeles, CA 90024.

A neurotropic virus, herpes simplex virus (HSV) wild type I, was used as a tracer in light and electron microscopic study of afferent and efferent fiber connections in the basal ganglia. Rats received microinjections (0.2-1µl) of highly concentrated HSV (10,000-40,000 plaque forming units per microliter) into the left neostriatum. Four days later substantial numbers of nuclear capsids and a few mature viruses were found in the nucleus and cytoplasm of many large neurons in the ipsilateral substantia nigra, largely in the zona compacta; in the midbrain dorsal raphé nucleus; in the medial thalamus; and in the frontal cortex bilaterally. The striatum itself showed few infected neurons. In another experiment when HSV was injected into the frontal cortex there was no virus spread to the neostriatum. The pattern of cell infection in these two experiments, plus prior knowledge of anatomical pathways, strongly suggests HSV is carried intraaxonally in a retrograde fashion in the experiments cited here.

In a third experiment, when HSV was injected into the left substantia nigra, a large number of virus labelled cells were seen in the ipsilateral neostriatum 5 days later. Most of the neurons infected by HSV were medium sized neurons measuring an average of 10-20µm in diameter. A parallel study in which the immunoperoxidase technique was employed following HSV injection into the substantia nigra confirmed the pattern of virus infection in the striatum.

99 PROSENCEPHALIC PATHWAYS RELATED TO THE AVIAN BASAL GANGLIA. Steven E. Brauth*, John L. Ferguson* & Cheryl A. Kitt* (SPON: D.I.Sommers) Dept. Psychol.,Univ of Md.,College Park,Md. 20742.

The avian basal ganglia include the paleostriatum augmentatum (PA), paleostriatum primitivum (PP), and nucleus intrapeduncularis (INP). Previous work indicated that PA was most comparable to the mammalian caudate nucleus, PP and INP to globus pallidus. The present study involves a series of anatomic experiments designed to clarify the afferent and efferent connections of these cell groups by means of horseradish peroxidase (HRP) histochemistry.

Injections of HRP into PA indicate that the source of telencephalic afferents to this structure is derived from a population of small to medium cells lying throughout the temporal-parietal-occipital area (TPO) and lateral cortical area (LCA) of the neostriatum. Some cells also appear to lie just medial to this region bounded by the dorsal archistriatal tract (DA). This result was demonstrated by both retrograde transport of HRP and by anterograde transport of the marker after injecting small quantities into the TPO area itself.

In addition to the above population of neurons, a small number of neurons in the medial part of PA project to the lateral portion. Both the medial and lateral portions of PA receive projections from cells in the TPO and LCA regions, however the lateral portion of PA receives a more extensive projection. The topography of the projection of TPO neurons onto PA is organized such that cells in the most ventral and lateral portions of TPO project upon lateral PA, and those lying dorsally and medially in TPO project upon medial PA. Nevertheless overlap in the projections of TPO and LCA neurons upon PA is present.

HRP injections in PP and INP labeled cells in PA. The majority of these retrogradely labeled cells were found in the lateral portion of PA and were scattered throughout this region. They constitute almost entirely the small cells of PA.

Subtelencephalic projections to PA are derived from the ventral portion of the nucleus tegmenti pedunculopontinus pars compacta (TPc). PP neurons receive projections from cells in the anterior nucleus of the ansa lenticularis (ALa) and in turn project extensively upon this thalamic cell group. ALa may thus resemble the mammalian subthalamic nucleus.

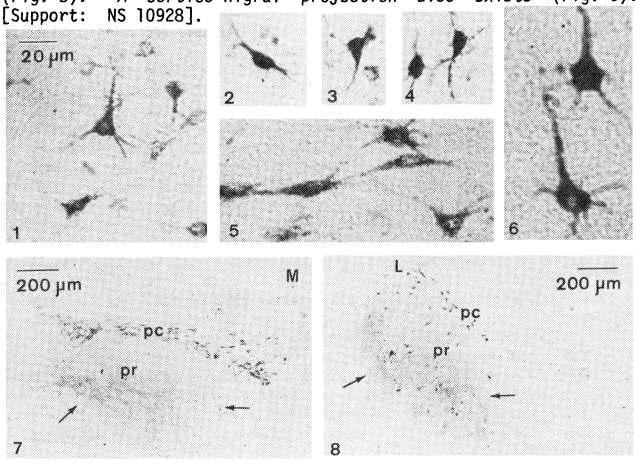
HRP injections confined to the TPO region of the telencephalon indicate that this region receives projections from the contralateral archistriatum pars ventralis (Av), and ipsilateral frontal neostriatum in areas just adjacent and also just dorsal to the ectostriatum (E). A small number of cells just ventral to PP also project into TPO ipsilaterally.

100 NIGRO-NEOSTRIATO-NIGRAL RELATIONSHIPS DEMONSTRATED BY HORSERADISH PEROXIDASE (HRP) HISTOCHEMISTRY. *LARRY L. BUTCHER*[1] *and GLENN J. GIESLER, Jr.*[2] Dept. Psychology[1,2] and Brain Research Institute[1], University of California, Los Angeles, CA, 90024, U.S.A.

HRP was infused into various regions of the rat caudate-putamen (CP) complex or substantia nigra (SN). 72 hrs later the animals were anesthetized and then sacrificed by transcardial perfusion with a glutaraldehyde-paraformaldehyde solution. After postfixation and sectioning the brains were processed according to the de Olmos o-dianisidine protocol for HRP, which demonstrates enzyme loci with greater sensitivity than other procedures for HRP. Accordingly, good cellular morphology can be obtained (Figs. 1-4: CP somata after intra-SN HRP injection; Fig. 5: pars compacta, pc, SN somata after intra-CP HRP injection; Fig. 6: cortical neurons after intra-SN HRP injection; 20 µm scale for Figs. 1-6).

Both anterograde and retrograde HRP transport were evinced consistently in our material (Figs. 7-8: SN after intra-CP HRP infusion; observe HRP both in somata in pc and in pars reticulata, pr, as well as in neuropil [arrows] in pr). Numerous CP somata, mostly in lateral regions of the nucleus, accumulated HRP after intra-SN enzyme injection. Medial (M) SN projects to medial CP, and medial CP projects to medial pr (Fig. 7). Similarly, lateral (L) SN projects to lateral CP, and lateral CP projects to lateral pr (Fig. 8). A cortico-nigral projection also exists (Fig. 6).

[Support: NS 10928].



101 AFFERENT CONNECTIONS OF THE GLOBUS PALLIDUS IN M. MULATTA AS DETERMINED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. K.W. Clausing*, M.E. Anderson and J.L. DeVito (SPON: O.A. Smith). Reg. Prim. Res. Ctr. and Depts. Rehab. Med. and Physiol. Biophys., Univ. Washington, Seattle, WA 98195

The high tonic discharge rates of globus pallidus neurons in awake monkeys make it probable that these neurons receive some potent excitatory synaptic input. Because most current electrophysiological evidence suggests that the major described pallidal afferent systems from the neostriatum and subthalamic nucleus are primarily inhibitory, we have used retrograde transport methods to identify possible additional sources of pallidal afferents.

The extent of the globus pallidus in M. mulatta was first mapped by recording the characteristic high frequency discharge of single pallidal units in awake animals. A silastic-filled stainless steel cylinder was stereotaxically implanted on the skull, and tungsten recording electrodes were inserted at a 30° angle through hypodermic cannulae into the putamen and globus pallidus. After the position of the globus pallidus was determined, a hypodermic cannula was inserted through the cortex to the outer or inner edge of the putamen and the needle of a microliter syringe was introduced through this into the globus pallidus. A 33% horseradish peroxidase (HRP) solution (0.2-0.3 µl) was injected in 0.1 µl increments and, after waiting 10-15 min, the needle was withdrawn through the cannula. The cannula was removed after an additional 30 min and proved an effective barrier to spread of HRP along the needle track.

In two animals with injections of HRP limited to the internal pallidal segment, retrograde label was seen in neurons of the ipsilateral peripeduncular pontine tegmentum, the dorsal raphe nuclei, the substantia nigra, the parafascicular nucleus, and the zona incerta. In one of the animals additional label was present in the putamen and subthalamic nucleus. A larger injection centered in the external pallidal segment, but extending medially into the internal segment and laterally into the putamen, resulted in label in the centromedian and medial pulvinar nuclei as well as in all areas listed above.

The fibers transporting HRP could be those terminating in the pallidum or damaged fibers passing through the pallidum and destined for the putamen. However, these data present the possibilities that (1) fiber systems destined for the neostriatum also have synaptic connections with pallidal neurons and (2) reciprocal synaptic connections exist between the globus pallidus and some of the sites to which pallidal axons project, such as the pedunculopontine nucleus.

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102 PROJECTIONS OF LOCUS COERULEUS AND RAPHE NUCLEI TO NEOSTRIATUM. James R. Couch and Glenn D. Goldstein.* Dept. Neurol., Kansas Univ. Sch. Med., Kansas City, KS 66103.

A series of 13 rats were employed to study projections from locus coeruleus (LC) and raphe nuclei to the caudatoputamen (CPU). Volumes of 2-10 µl of a 10% solution of horseradish peroxidase (HRP) (Sigma type VI) were injected into the anterior part of the right CPU employing a Gilmont microsyringe (accuracy 0.1 µl ±.04%). After 24 hrs the animals were anesthetized with with diabutal and perfused with a solution of 10% formaldehyde - 1.25% glutaraldehyde followed by 5% sucrose. Sections were cut serially at 30 microns, mounted, and incubated in 0.03% 3,3 diaminobenzidine followed by exposure to H_2O_2 and 3,3 diaminobenzidine. As controls, a sham injection into the cortex and an intravenous injection of 12 mg of 10% HRP were employed. Neurons projecting to the CPU were identified as polygonal structures with golden brown granules of reaction product in the cytoplasm.

In 7 animals given 5-10 µl injections, substantial numbers of labelled neurons were seen in both right and left LC. The labelling showed a modest ipsilateral preponderance compared to the contralateral side. On both sides labelling was somewhat heavier in the caudal, compared to the rostral LC. Ipsilateral labelling of substantia nigra (SN) neurons was seen in all animals. With 5-10 µl injections there was some spread of HRP beyond the CPU. Six additional animals were tested with 2-3 µl injections with which the HRP remained localized to the CPU. In 4 animals, there was labelling in the SN and in these, there was also labelling in the LC bilaterally. The labelling was more sparse but followed the pattern noted above. In 2 animals there was no SN and also no LC labelling.

With 5-10 µl injections, there was a modest amount of labelling in the dorsal raphe nucleus (DRN) and occasional labelling in nuclei raphe pontis and magnus. These findings were confirmed with 2-3 µl injections. No labelling of the median raphe nucleus was seen. These findings are in accord with other reports. No labelling of LC, DRN or SN was seen in control animals.

The LC, the most concentrated collection of norepinephrinergic neurons in the brain, has extensive projections, but LC-to-CPU connections have not previously been identified. The findings presented suggest there are substantial homolateral and contralateral projections from LC to CPU. The CPU contains a small but significant concentration of norepinephrine and the proposed LC-to-CPU pathway may account for much of the norepinephrine in the CPU.

103 POSTNATAL DEVELOPMENT OF THE NEOSTRIATUM IN MONKEYS (M. MULATTA). Marian DiFiglia, Tauba Pasik and Pedro Pasik. Dept. of Neurology, Mount Sinai School of Medicine, CUNY, New York, N.Y. 10029.

Paired specimens of the neostriatum of monkeys at 0, 1, 2, 4, 8, 12 weeks and 3-4 years of age were prepared for Golgi and electron microscopic studies. Wide variations in the degree of maturity of each previously described neuronal type (DiFiglia et al., Brain Res., 1976, 114, 245) are observed within the same specimen at 0 and 1 week. Some cells are not differentiated enough to be classified. General immature features of dendrites include growth cones with filopodia, other enlargements and long filiform processes. The spiny I neuron exhibits dendritic varicosities which decrease in frequency with age, and are only occasionally seen at terminal portions in the adult. The least mature cells have few dendritic spines mostly of the thin type. The most mature cells at each period show a progressively greater number of spines but their relative distribution and types (thin, stubby, mushroom) remain about the same. The spine density increases from a mean of 8.6 spines per 10µm of dendrite length at birth to 14.1 at 8 weeks, reaching maximal levels of 15-17 by 12 weeks. Concurrently, the dendritic field radius grows from 100 µm to 200 µm. The long axon of these neurons shows varicosities up to 4 weeks, and acquires mature features by 8 weeks. Beaded collaterals present from birth, continue to elaborate with age. The spiny II neuron at early ages has a dendritic field radius of up to 200 µm which may increase to 600 µm at 4 weeks. The spine density is characteristically higher at birth (7 per 10 µm) and decreases progressively down to 5 per 10 µm at 8 weeks. The long axon and its extensive collateral system are well developed by 1 week although bulbous enlargements are noted. The 3 types of short axon aspiny neurons exhibit immature dendritic features, most notably spine-like and filiform processes. Axonal arborizations vary from immature to well developed at 0 and 1 week. Four categories of presumably afferent axons are already present at birth and include: 1) thick axons branching extensively into undulating, terminal processes; 2) thick axons having grapelike clusters of large terminal boutons; 3) axons of intermediate diameter with numerous varicose branches often terminating in axonal growth cones with filopodia; and 4) thin,straight processes exhibiting short appendages with terminal boutons. All of these afferents can be correlated with similar types found in the adult.

Electron microscopic examination of striatal neuropil confirms the presence of immature dendritic and axonal features at early ages. At birth, both immature and mature synapses occur on the dendrites of aspiny and spiny (spines and shaft) neurons. Axonal profiles with features associated with dark degeneration in the adult are seen up to 4 weeks. Aided by N.I.N.C.D.S. Grant # NS-11631.

104 REVERSE TOLERANCE TO AMPHETAMINE OF MICE BEARING UNILATERAL ELECTROTHERMAL STRIATAL LESIONS: EFFECT UPON CIRCLING RESPONSE TO APOMORPHINE. Stanley D. Echols. Dept. Pharmacol. Merrell-National Laboratories, Cincinnati, Ohio 45215.

Mice bearing unilateral striatal lesions circle ipsiversively when administered amphetamine. It has previously been shown in this laboratory that such responses increase upon repeated administration of the same dose. This experiment was to determine whether such reverse tolerance to amphetamine affects the circling response to apomorphine. Mice were electrothermally lesioned, administered apomorphine, 0.4 mg/kg s.c., and circling was counted minutes 2-10 afterwards. Good responders were divided into two groups of ten matched according to response. One group received 4 doses of d-amphetamine, 4 mg/kg s.c., at weekly intervals; the other group received corresponding saline injections. The mean ipsiversive turns over 1 hour was 370 after the first dose of amphetamine and 644 after the fourth ($P < .01$), demonstrating reverse tolerance. The mean response of that group of mice to apomorphine, 0.4 mg/kg s.c., had been 33 before the amphetamine injections; afterwards it increased to 49 ($P < .01$). In the other group of mice, which had responded to the earlier apomorphine injection with 34 turns, the response after the saline injections decreased to 12 ($P < .01$). Hence, reverse tolerance to amphetamine is reflected in the response to apomorphine. Reverse tolerance to amphetamine therefore seems unlikely to be mediated solely by presynaptic changes of the nigro-striatal dopaminergic system.

105 THE ROLE OF NIGRO-TECTAL PROJECTIONS IN RELATION TO TECTO-SPINAL TRACTS AND HEAD TURNING. James E. Faber & Donald H. York, Dept. of Physiol., Sch. of Med., Univ. of Missouri, Columbia, Mo., 65201.

The anatomical projection of neurons from the substantia nigra to the superior colliculus has been demonstrated by several groups of workers. The role that this pathway may play in 1) relaying information from the basal ganglia to descending spinal cord tracts or 2) tectal integration of other inputs, is the subject of this investigation. Single unit microelectrode recordings were undertaken from the superior colliculus in male Sprague-Dawley rats (300-650 gms) anesthetized with chloral hydrate or urethane. Cells were examined in all layers of the colliculus for input from the substantia nigra (SN), neck muscles (NM), visual system (VS) and spinal cord (SC). Cells in the more superficial layers characteristically responded to visual input whereas other inputs were more diffusely spread throughout the colliculus. Of 228 neurons evaluated in the superior colliculus, 57 were antidromically identified by nigral stimulation and followed stimulus frequencies over 200/sec. About half of these tecto-nigral cells were activated by a light flash delivered to the eyes and 10 cells also received neck muscle input. In contrast, orthodromic activation of cells in the superior colliculus was obtained upon either stimulation of SN, pars reticulata (6 out of 77 cells) or SN, pars lateralis (29 out of 42 cells). Cells evoked by stimulation of the latter area (pars lateralis) were also excited by photic activation (19 out of 42) and neck muscle input (6 out of 13). Stimulation of the ventral funiculus of the cervical spinal cord also evoked these latter type of units at constant latency (~4 msec) and followed stimuli over 200/sec. Thus, of 16 tecto-spinal identified cells, all were activated by the substantia nigra (pars lateralis). These findings suggest that the superior colliculus may relay basal ganglia output to neck motoneurons in the cervical cord which results in the characteristic head turning movements and circling observed with stimulation of a variety of basal ganglia structures.

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106 COBALT INJECTIONS INTO THE SUBSTANTIA NIGRA OF THE RAT: EFFECTS ON BEHAVIOUR AND METABOLISM OF STRIATAL DOPAMINE. Ruggero Fariello, Masato Shibuya*, Irene J. Farley*, Kathleen S. Price*, Kenneth G. Lloyd and Oleh Hornykiewicz. Dept. Psychopharm., Clarke Inst. Psych., Univ. Toronto, Toronto, Canada.

Small amounts of cobalt (5-12 µg in 0.5 µl) unilaterally injected into the substantia nigra (SN) of rats caused a strong, spontaneous contralateral turning which was suppressed by haloperidol. One to two days after intranigral cobalt microinjection, d-amphetamine decreased the intensity of the contralateral turning; five days after cobalt application, d-amphetamine changed the direction of the turning to the ipsilateral side. Intranigral cobalt microinjection initially caused a significant increase in the concentration of dopamine (DA), 3,4 dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the ipsilateral striatum. Thereafter, the levels of DA and its metabolites declined progressively to reach, 4 days after cobalt, values significantly below the control levels. Similar biochemical changes in the striatum were also seen after partial electrolytic lesions of the SN. However, in contrast to cobalt, such SN lesions produced only mild contralateral turning. Frontal hemisection completely interrupting the nigrostriatal DA pathway did not result in any increase of striatal DOPAC or HVA levels, and produced no significant turning. Thus, there was no apparent parallelism between the intensity and time course of the turning behaviour induced by the different procedures and the changes in DA metabolism in the striatum. However, within the cobalt injected SN the levels of GABA and glutamic acid decarboxylase (GAD) were decreased to 40% of the contralateral control values. The possibility is considered that the depression of a GABA mediated inhibitory influence upon the nigrostriatal or mesolimbic DA neurons may play a role in the cobalt-induced contralateral turning as well as altered striatal DA metabolism.

107 MOTOR CORTEX PROJECTIONS TO THE CAUDATE NUCLEUS. I. ELECTROPHYSIOLOGY. E. Garcia-Rill, C.D. Hull and N.A. Buchwald, MRRC, Sch. of Med., UCLA, Los Angeles, CA. 90024.

Dense afferent inputs from the frontal cortical areas have been described to project in a topographical manner onto the striatum. The experiments reported here were designed to reveal any possible differential projections to the caudate nucleus from different areas within the motor cortex. Four-pronged stimulating combs were placed within the gray matter bilaterally, spanning the precruciate cortex of the cat. The medialmost lead was generally 2 mm from the midline and within area 6aβ and the most lateral lead was usually 3 mm beyond the end of the cruciate sulcus within area 4γ. These two areas are known to correspond with the representation of the trunk or axial musculature medially, and the representation of the forelimb musculature laterally.

Intracellular recordings using potassium citrate-filled micropipettes were made from the caudate nuclei (coordinates A 18-15) of locally-anesthetized, paralyzed cats. Over 95% of cells showed EPSP-IPSP sequences, and the rest had only IPSP responses. Latencies to onset of postsyaptic potentials (PSPs) for the large majority of cells (88%) were between 5 and 20 msec (range 4-34 msec), with a mean rise time (onset to peak of PSP) of 13.4 msec (range 4-22.5 msec).

However, 72% of caudate neurons responded preferentially to stimulation of the ipsilateral medial precruciate cortex. Only 9% of cells responded preferentially to stimulation of the ipsilateral lateral precruciate area, while 7% responded best to stimulation of the contralateral medial precruciate cortex and 12% showed no response to cortical stimuli. Preferential responses involved lower threshold (usually 90-250 µA), shorter latency, higher amplutide PSPs, and the ability of the preferred input to suppress conditioning shocks to other areas during the rising phase of IPSP, and in turn not be suppressed during the rising phase of IPSPs elicited by stimulation of other leads.

Histological verification of the position of cells recorded showed that inputs from the medial precruciate area are distributed on the ventral and lateral parts of the caudate. The lateral precruciate cortex elicited preferential responses in cells located in a small region of the ventrolateral caudate nucleus. The majority of non-responsive cells were found dorsally, and those responding to the contralateral medial precruciate cortex were located in the center of the caudate nucleus.

The ipsilateral medial precruciate cortex, corresponding to the motor representation of the axial musculature, showed a potent and widespread input to the caudate nucleus. Within this projection, circumscribed regions of the caudate demonstrated preferential input from the ipsilateral lateral and contralateral medial precruciate cortex. Supported by USPHS Grants HD-05958 and MH-7097.

108 CORPUS STRIATAL (PALEOSTRIATAL COMPLEX) INTERVENTIONS AND STEREOTYPED BEHAVIORS IN PIGEONS. Irving J. Goodman and Robert E. Stitzel*. Depts. of Psychology and Pharmacology, West Virginia University, Morgantown, WV 26506.

Stereotyped pecking may be induced in pigeons by apomorphine, a dopamine agonist, and blocked by Haloperidol, a dopamine antagonist. The paleostriatum augmentatum, a component of the pigeon's corpus striatum, contains a relatively high level of dopamine. The present study attempted to determine the role of this forebrain structure in stereotyped behavior by combining several experiments: (A) Pigeons with chronically implanted stimulating electrodes within the paleostriatum provided an opportunity for tests of electrically evoked responses. Among the varied evocable responses from this structure were stimulus bound floor pecking and circling, separable responses which were usually directed contralateral to the stimulated side. These effects were observed consistently over months of testing; (B) Pigeons, acutely prepared under ether and thus quickly revived, had apomorphine (50 ug/2 ul) unilaterally administered directly to the paleostriatum. This treatment produced contralateral turning and pecking for a short period (2 - 10 min); (C) Correspondingly, unilaterally lesioned paleostriatal pigeons exhibited apomorphine-induced (2.0 - 3.5 mg/kg, IP) pecking and circling (when it occurred) markedly and consistently toward the lesioned side (30 - 60 min sessions). These effects continued through 6 months of testing; (D) Bilaterally lesioned paleostriatal pigeons exhibited a transient reduction or absence of apomorphine-induced stereotyped responding which lasted up to 10 days postoperatively. While the intact corpus striatum provides a structure from which stereotyped pecking and circling may be initiated by electrical or chemical stimulation and sustains a functional imbalance of stereotyped behavior in the unilaterally lesioned preparation, the bilaterally lesioned preparationshows only a short term disruption of apomorphine -induced stereotyped responding. These results suggest that the critical components of the total mechanism for apomorphine-induced stereotyped responding are probably located lower in the brain, within the brain stem.

109 INTERACTION BETWEEN GABA, DOPAMINE AND SUBSTANCE P IN THE RESPONSE OF NIGRO-STRIATAL SYSTEM TO ANTIPSYCHOTICS. A. Guidotti and K. Gale*. Lab. Preclinical Pharmacology, NIMH, St. Elizabeths Hospital, Washington, D.C. 20032.

Measurement of striatal tyrosine hydroxylase (TH) in the presence of subsaturating amounts of pteridine cofactor was taken as an index of activity of nigro-striatal dopamine (DA) neurons. The activation of striatal TH induced by systemic injection of neuroleptic drugs (haloperidol or chlorpromazine, 0.1-1 mg/kg) was abolished by: 1) systemic administration of DA receptor agonists (apomorphine and Trivastal); 2) systemic injection of GABA-mimetic drugs (muscimol and diazepam); 3) discrete unilateral electrolytic lesions placed anterior and lateral to substantia nigra (SN). These electrolytic lesions reduced levels of GABA and Substance P (SP) in SN by 70-90% and abolished DA-sensitive adenylate cyclase in SN. Our evidence,based on measurements of DA-sensitive adenylate cyclase and DA receptor binding, demonstrates that DA receptors in nigra are not located on DA neurons and are therefore not autoreceptors. Instead, DA receptors in SN are present on terminals of striato-nigral fibers (GABA or SP). To determine the role of these nigral DA receptors, apomorphine (10^{-9}-10^{-6}M),or haloperidol (10^{-9}-10^{-6}M) was injected into SN in rats pretreated with saline or with systemic haloperidol (0.15 mg/kg i.v.). These intranigral treatments did not significantly modify the kinetic state of striatal TH indicating that DA receptors in nigra do not constitute the critical site of neuroleptic action with respect to striatal TH. On the other hand, activation of GABA receptors in SN by intranigral muscimol (0.3 nmoles) injection blocked the neuroleptic-induced activation of striatal TH.

These experiments suggest a role (1) for striatal postsynaptic DA receptors and (2) for nigral GABA receptors,in the neuroleptic induced activation of striatal TH. We have previously demonstrated the presence of respectively independent GABA and SP neurons projecting from striatum to nigra (Fed. Proc. 30: 394, 1977). It is likely that nigral SP also may be involved in the action of neuroleptics. This is suggested by the inability of GABA antagonists alone (isoniazid, bicuculline, picrotoxin injected systemically or intranigra) to activate striatal TH.

110 ANTIDROMIC ACTIVATION OF DOPAMINERGIC AND OTHER NEURONS IN THE NIGROSTRIATAL PATHWAY. P.G. Guyenet☆ and G.K. Aghajanian, Depts. Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508

The purpose of the present study was to identify nigrostriatal dopaminergic neurons by antidromic (AD) stimulation. Rats were anesthetized with chloral hydrate (400 mg/kg) and the single unit activity of neurons located in the substantia nigra (SN) was recorded while stimulating various sites in the caudate-putamen (CP) (bipolar electrodes, rectangular pulses 500 μsec, 0.3 to 2 mA). A successful search for AD-activated cells depended on the prior determination of the topography of the nigrostriatal projection; the technique of retrograde transport of horse-radish peroxidase (HRP) was used for that purpose. AD-activated cells (meeting the requirements of fixed latency and positive collision test) were found only in the area of the SN topographically related to the locus in the CP which was stimulated. The vast majority of these cells corresponded to the type described previously by Bunney et al. (1973) as being the DA-containing neurons of the nigrostriatal pathway; they are the majority of the spontaneously active cells of the SN pars compacta, have a slow bursting pattern of discharge < 7 spikes/sec, a wide action potential of 2 to 4 msec and are inhibited by low doses of the DA agonist apomorphine (APO) (10 to 40 μg/kg, i.v.). The slow conduction velocity of these neurons (0.3 to 0.6 m/sec) is characteristic of unmyelinated axons. These slow-conducting APO-sensitive neurons could also be AD-activated by stimulating the medial forebrain bundle (MFB). Many other types of neurons in the SN could also be activated by this procedure, but with much shorter latencies (0.8 to 2.9 msec instead of 4.5 to 7.5 msec). The injection of 6-hydroxydopamine (a neurotoxic agent relatively specific for catecholaminergic neurons) above the MFB (2 μl, 6 μg) selectively blocked AD-activation of the slow-conducting APO-sensitive neurons of the SN pars compacta, providing further evidence that these are the DA-containing neurons. A small minority of cells with electrophysiological characteristics completely different from the DA neurons could also be AD-activated by stimulating various areas in the CP (500 μsec pulses, 0.3 to 1.0 mA). These cells are located mainly near the ventral border of the SN pars compacta, are not inhibited by APO, have a conduction velocity of 2-3 msec and a firing rate of up to 25 spikes/sec. In conclusion these results indicate that 2 types of neurons contribute to the nigrostriatal pathway; 1) DA neurons; slow conducting and APO-sensitive, they are the most commonly encountered in the SN pars compacta; 2) fast conducting APO-insensitive neurons; less numerous, they are located in or close to the SN compacta. (USPHS Grant MH-17871, The State of Connecticut and the CNRS, France).

111 TRIGEMINAL INFLUENCES ON CAUDATE AND SUBSTANTIA NIGRA UNITS. J.A. Harper* and T. I. Lidsky (SPON: J.S. STAMM). Dept.Psych SUNY at Stony Brook, NY 11794

Trigeminal influences upon the caudate nucleus and substantia nigra were assessed in flaxidilized, chloralose anesthetized cats. Electrical stimulation was applied to two loci involved in jaw movement reflexes. Afferents from periodontal mechanoreceptors involved in reflex jaw opening were stimulated via electrodes in the inferior dental nerve (IDN). In addition, afferents from spindle receptors in jaw elevator muscles were stimulated via electrodes in the trigeminal mesencephalic nucleus (MES 5). MES 5 stimulation evokes jaw closure. A significant proportion of units in both caudate (MES 5-54%, IDN-50%) and Substantia nigra (MES 5-66%, IDN-55%) were affected by IDN and MES 5 stimuli. Both ipsilateral and contralateral stimulation were equally effective in evoking unit responses. Patterns of response to MES 5 and IDN stimulation in neurons receiving convergent input were similar; however, latency differences of unit responses served to differentiate stimulation loci.

Although the striatum is generally thought to be the major source of afferents to the substantia nigra, latency considerations suggest that most trigeminal responses recorded in the nigra are not conveyed via caudato-nigral connections. Latencies of caudate responses (mean latencies: MES 5-36 msec, IDN-48 msec) were longer than responses recorded in the nigra (mean latencies: MES 5-20 msec, IDN-28 msec). Indeed the shortest latencies of responses in the caudate (16 msec) were longer than many of the responses recorded in the substantia nigra.

Tactile stimulation of the face and mouth was also tested. Many basal ganglia neurons have peripheral receptive fields. These fields are large and often encompass two or three branches of the trigeminal nerve. (Supported by U.S.P.H.S. Biomed. Sci. Support Grant 5 So5 RR07067 to SUNY at Stony Brook).

112 MORPHOLOGICAL STUDIES ON THE POSSIBLE GLUTAMATERGIC CORTICO-STRIATAL PATHWAY. T. Hattori, E. G. McGeer, P. L. McGeer
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The autoradiographic tracing technique was combined with intrastriatal injections of the neurotoxin kainic acid (KA) to provide morphological evidence bearing on the recent postulate that the cortico-striatal pathway is glutamatergic in nature. ^{3}H-Proline (20 μC) was distributed by six injections throughout the prefrontal cortex. Twenty four hours later, the distribution of axonally transported proteins in the striatum was studied by light and electron microscopic autoradiography. In some animals, degeneration was induced by an ipsilateral intrastriatal injection of KA (1.25 nm in 0.5 μl saline) ten hours before sacrifice. At the light microscopic level, the label appeared to be rather uniformly distributed in the control striatum and in unaffected areas of the KA-injected striatum. In all of the area affected by KA, grains appeared in either the same number per mm as in the unaffected areas or in patches showing much greater grain density. These clusters of grains resembled those reported in immature animals.

At the electron microscopic level, the labeled boutons in the control striatum had fairly packed round vesicles of 40-45 nm in diameter and made clear asymmetrical synaptic contacts with dendritic spines. In the KA-affected area, most of the labeled boutons made direct synapses with degenerating dendritic elements. However, the vesicle density in the labeled boutons (approximately 180 per μm^2) was the same whether they had synaptic contacts with normal or degenerating dendrites.

These results indicated that: 1) Intrastriatal injections of KA did not produce in 10 hours morphological changes in the presynaptic cortico-striatal terminals nor did it reduce the amount of axonally transported label accumulated in these terminals. In fact, the appearance of densely labeled clusters suggests that the degeneration of postsynaptic elements may have stimulated transport and/or accumulation of label in the presynaptic elements. 2) KA in 10 hours did induce massive degeneration of cell soma and dendrites in the striatum and radioactive macromolecules axonally transported in the cortico-striatal path preferentially labeled boutons in synaptic contact with KA-sensitive dendrites. In view of the evidence that this path is glutamatergic, this provides some morphological support for the hypothesis that neuronal elements carrying many glutamate receptors may be particularly sensitive to the toxic effects of KA.

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113 IMPORTANCE OF THE SUBSTANTIA NIGRA IN ROTATIONAL BEHAVIOR OF RATS. Gordon K. Hodge and Larry L. Butcher. Depts. Psych., U. of New Mexico, Albuquerque, NM 87131, and UCLA, Los Angeles, CA 90024.

Many variables can affect the elicitation and assessment of rotational behavior, and since many of these factors are uncontrolled, interpretations of data and reconciliation of theories derived from such data is difficult (see Glick et al., Life Sci., 1976, 18, 889). One variable responsible for some confusion is lesion placement and extent. Our data demonstrate that relatively subtle differences in lesion placements can result in profound differences in the manifestation of rotational behavior.

Rotational behavior following unilateral radio-frequency (RF) or 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra or median raphe was recorded in computerized photocell devices which counted rotations as the rat traversed an uninterrupted 360° turn around a central column. Spontaneous rotations were recorded for 7 days postoperatively. The effects of D-amphetamine (AMP; 1.5, 5.0 mg/kg) and apomorphine (APO; 1.0 mg/kg) were then noted.

The asymmetries displayed by rats varied considerably. Only rats with RF lesions of pars compacta (PC) or the raphe exhibited spontaneous rotations (contralateral). Following drug treatments, rats with 6-OHDA lesions pivoted in a very constricted manner, often wrapping themselves around the central column; animals with RF lesions typically rotated in broader circles about the column. All drugs potentiated contralateral turning in rats with asymmetrical lesions of the raphe area, but histological data indicated that the lesions involved tissue encompassing the raphe as well. Ipsilateral turning was induced by 5.0 mg/kg AMP in RF lesions of PC but APO was without effect. Both doses of AMP led to ipsilateral turning in rats lesioned with 6-OHDA, and APO to contralateral turning. Contralateral rotations to APO were also seen in rats with large lesions of the tegmentum which spared much of PC. Ipsilateral turning occurred with lesions of the medial lemniscus and adjoining areas or with lesions of pars reticulata.

Although PC appears involved in the mediation of rotational behavior, the nature of this involvement is not as straightforward as previously assumed on the basis of techniques which probably encroached upon other systems capable of affecting motor symmetry. The degree of spontaneous contralateral turning shown by rats with PC lesions seemed related to the extent of PC damage, but responses to drugs did not. Although AMP elicited ipsilateral rotations, neither quantitative nor qualitative effects of the drug appeared contingent upon the degree of PC injury. Disparities between our results and those of others are attributable to differences in lesion size and placement per se; these factors appear independent of the manner in which the lesions were made.

114 DIFFICULTY IN INITIATING ARM MOVEMENTS DURING COOLING OF THE PUTAMEN IN THE MONKEY. J. Hore and T. Vilis. Dept. Physiology, Univ. Western Ontario, London, Ontario, Canada N6A 5C1.

It was previously reported (1) that cooling in the region of the globus pallidus of Cebus monkeys did not produce a delay in the onset of prompt flexion or extension elbow movements in response to a visual GO signal, although it produced movements with decreased amplitudes and velocities. This same paradigm was used to investigate motor performance of 3 Cebus monkeys in which cooling sheaths were implanted in the ventral portion of the rostral putamen. Cooling the putamen sheath to 10°C for 10 min produced no major change in simple reaction time of the contralateral arm, as measured both to EMG and to movement onset, although such cooling usually produced a decrease in velocity of movement, due to a decrease in the phasic activity of the agonist muscle and an increase in activity of the antagonist.

However, severe putamen cooling (5° sheath ref. temp. for greater than 10 min) produced, in one monkey, a remarkable motor impairment, which was characterized by an increase in movement reaction time of up to 200 msec. Inspection of such trials revealed that there was no EMG activity in the agonist at the time that it would have occurred in the controls, but that there was a large burst of activity in the antagonist muscle shortly after this time. The result of this antagonist activity was that the monkey moved initially in the wrong direction. This incorrect movement was terminated about 100 msec after onset by movement in the correct direction. When the agonist muscle was loaded so as to increase its tonic level of activity, a small burst of EMG activity now occurred in the agonist in some cooling trials, at the same time after the GO signal as occurred in controls. This suggests that the movement disorder was due to inappropriate EMG activity in response to correctly timed descending signals.

The results from these 3 monkeys are consistent with the recent conclusion of Denny-Brown (2) that one function of the basal ganglia is to provide a preparatory facilitation or "set" in initiation of movement. Thus failure to prepare motor cortex or spinal cord for a forthcoming movement during basal ganglia dysfunction, may cause a correctly timed movement initiation command to be decoded into an inappropriate muscular response.

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115 EFFECT OF KAINIC ACID LESIONS IN THE CAUDATE NUCLEUS ON MUSCARINIC CHOLINERGIC RECEPTORS: CORRELATION WITH HUNTINGTON'S DISEASE. R.E. Hruska*, J.T. Coyle, R. Schwarcz* and H.I. Yamamura (SPON: W.D. Barber). Univ. of Arizona Health Sciences Center, Tucson, AZ 85724 and Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The degeneration of neurons in the rat caudate nucleus after kainic acid injections resembles the neurochemical findings in the caudate nuclei of postmortem samples from patients with Huntington's Disease. Accordingly, we measured muscarinic cholinergic receptor binding in kainic acid-lesioned and control caudate nuclei using the muscarinic antagonist, [^{3}H]3-quinuclidinyl benzilate ([^{3}H]QNB). Kainic acid (2 µg in 1 µl) was injected unilaterally into the caudate nucleus. Five days after injection, the rats were sacrificed and the lesioned and contralateral, non-lesioned caudate nuclei were removed. The tissue was homogenized and washed twice before receptor binding assays were performed. We evaluated the effect of kainic acid lesions on the apparent dissociation constant (K_D) and on the total number of binding sites (Bmax). The K_D values were 11 to 13 pM for the lesioned and non-lesioned caudate nuclei, respectively, as determined from the rate of association (7×10^8 $M^{-1}min^{-1}$ for both lesioned and non-lesioned caudate nuclei) and from the rate of dissociation (9×10^{-3} min^{-1} for the lesioned and 8×10^{-3} min^{-1} for the non-lesioned caudate nuclei). The apparent K_D was also determined from saturation isotherms. The analyses of the saturation curves by Scatchard plots revealed a K_D value of 20 to 50 pM for both the lesioned and non-lesioned caudate nuclei. Therefore, the kainic acid lesions have no significant effect on the apparent K_D value. Similar findings have been reported in Huntington's Disease. However, the kainic acid lesions do decrease markedly the density of specific binding sites (Bmax), as determined from Scatchard plot analyses. The non-lesioned caudate nuclei had 1.33 ± 0.15 picomoles of [^{3}H]QNB bound per mg protein, while the lesioned caudate nuclei had 0.82 ± 0.12 picomoles of [^{3}H]QNB bound per mg protein, which is a 40% reduction in the total amount of specific binding. The decrease in [^{3}H]QNB binding effected by kainic acid lesions of the caudate nuclei parallels the decrease in [^{3}H]QNB binding found in Huntington's Disease caudate nuclei (∿50% reduction in [^{3}H]QNB binding). Since kainic acid lesions do not alter the apparent K_D value but do decrease the density of binding sites, as observed previously in Huntington's Disease, these findings strengthen the usefulness of kainic acid lesions of the caudate nuclei as an animal model for this disease. (Supported by NIH grants MH-27257 and NS-13584, fellowship NS-05585 to R.E. Hruska, and RSDA to J.T. Coyle and H.I. Yamamura.)

116 MICROIONTOPHORETIC STUDIES ON GLOBUS PALLIDUS NEURONS OF MACACA MULATTA MONKEY. Ronald D. Huffman, Leslie P. Felpel and Robert L. Polzin. Dept. Pharmac., The Univ. of Texas Health Sci. Center, San Antonio, Texas 78284.

A number of substances that are strong candidates for neurotransmitters in the mammalian CNS are found in high concentrations in the basal ganglia. These include acetylcholine (ACh), dopamine (DA), serotonin (5HT), γ-aminobutyric acid (GABA), substance P and taurine. Little is known about the effect of these substances and other putative neurotransmitters on the responsiveness of neurons of the globus pallidus. In this investigation the responsiveness of primate pallidal neurons to microiontophoretically applied (7 barrel glass micropipettes) putative neurotransmitters was studied. Macaca mulatta monkeys anesthetized with chloralose-urethane were used as the experimental animal. Pallidal neurons characteristically discharged spontaneously with rates of 150-200 spikes/sec being fairly common. Eighty pallidal cells were studied; of these 15 were located in the external pallidal segment and 6 were located in the medullary lamina between the two segments. The number of cells studied with each of the putative neurotransmitters is given in the following Table:

GABA	Glyc	Taur	Glut	DA	NA	5HT	ACh	Sub P
74	38	30	50	25	6	9	47	12

GABA, glycine and taurine depressed the spontaneous firing of neurons located in both pallidal segments. GABA was a powerful depressant and stopped spontaneous firing of many neurons with currents less than 5 nA. Only 2 of 74 pallidal cells tested did not respond to GABA. Cells located in the internal pallidal segment were generally more responsive to the depressant action of GABA than cells located in the external segment or laminar region. In general, glycine was not as effective a depressant as was GABA and currents 2-3 times those used to eject GABA were required with glycine to produce a depression of pallidal firing equivalent to that produced by GABA. Taurine was a weak depressant and many pallidal cells did not change their responsiveness in the presence of taurine. L-glutamic acid was a weak excitant and failed to excite about 50% of the pallidal neurons to which it was applied. ACh was a weak to moderate excitant of most pallidal cells although a few cells were unaffected by ACh. DA was also a weak excitant and produced a delayed and prolonged excitation of many pallidal neurons. GABA frequently caused a gradual reduction in spontaneous firing following its application; DA characteristically increased the responsiveness of the cells and restored the spontaneous firing rate to the pre-GABA level. Noradrenaline, 5HT and substance P had no effect on the responsiveness of any pallidal neurons studied. (Supported in part by NIH Grant NS-10602, USPHS.)

117 A TECHNIQUE FOR RECORDING SINGLE-UNIT ACTIVITY IN UNANESTHETIZED, RESTRAINED RATS DURING MOVEMENT. Lawrence R. Huntoon. Dept. Physiol., L.S.U. Med. Ctr., New Orleans, La. 70112.

A technique has been developed which permits single-unit recordings in restrained, unanesthetized rats during movement. Unlike other techniques, this method allows multiple microelectrode penetrations to be made in prescribed stereotaxic planes. In this manner, stereotaxic exploration of a given area can be performed on a daily basis and data can be collected over a period of several weeks. The essential features of the technique are: 1. Non-traumatic rigid head restraint 2. Body restraint which allows movement of limbs 3. Training rats to bar press by employing the operant technique of intracranial self-stimulation (I.C.S.S.).

Non-traumatic head restraint is accomplished using a 3/8" plexiglass block tailored to fit adult rats' heads, with a rectangular window to allow microelectrode penetrations. Small screws at the corners of the headpiece serve to rigidly fix the animal's head in a conventional stereotaxic frame for recording sessions, and between sessions they are used to secure a 1/16" plexiglass cap over the window. Initial surgery involves drilling a rectangular portion of the skull away and cementing the headpiece in the stereotaxic horizontal plane such that the plexiglass window is in position over the exposed area. Precautions are taken to protect dura and underlying cortex.

Appropriate body restraint is obtained using an ANIMAL HOLDER (C.H. Stoelting Co.). This restraining apparatus allows considerable freedom of movenent of body forelimbs and hindlimbs. With the addition of a bipolar electrode placed in the medial forebrain bundle (MFB) rats can be trained to bar press (I.C.S.S.) while restrained with either forelimb or hindlimb. An unexpected advantage of I.C.S.S. conditioning is the finding that following a single 2 hr. training session, rats can be placed in the restraining device without struggling or other overt signs of stress. Therefore, data can be obtained under relatively non-stressful conditions of a quality which would not exist under conditions of restraint alone.

Stainless steel microelectrodes are currently being used with this preparation in an attempt to correlate limb movement with single-unit activity in rat substantia nigra (SN). In addition to the MFB electrode, five other stainless steel bipolar electrodes are placed in the caudo-putamen to permit electrophysiological identification of cells being studied in SN. Preliminary results indicate that cells exist in rat SN which are movement related. It is concluded that this technique can be used successfully to record single-unit activity in unanesthetized restrained rats during movement, and that combined use of I.C.S.S. permits data aquisition under relatively non-stressful conditions.

118 CONDUCTION PROPERTIES OF CAUDATE EFFERENT AXONS IN THE CAT. J.D. Kocsis*, C.P. VanderMaelen*, and S.T. Kitai (SPON: G.D. Schoener). Morin Memorial Lab., Dept. Anat., Sch. Med. and Dept. Psychol., Wayne State Univ., Detroit, Mich. 48201.

Antidromic action potentials were recorded intracellularly from caudate (Cd) nucleus neurons in cats anesthetized with α-chlorolose after stimulation of the substantia nigra and the internal capsule. The antidromic nature of these spikes was determined by collision of antidromic spikes with intracellularly induced orthodromic spikes. The recorded neurons were identified as spiny neurons with intracellular injections of horseradish peroxidase. With double antidromic stimulation, the second spike shows a decrease in latency if it is induced up to 200 msec after the conditioning spike. The maximum latency shift is about 10% of the control latency. The magnitude of the latency shift is cumulative with conduction distance, suggesting that a change in conduction velocity occurs along the axon. Conditioning orthodromic spikes, either synaptic or direct, also induce a latency shift of test antidromic spikes. The latency shift is independent of somatic polarization levels and only occurs when the test antidromic spike is preceded by an action potential. Threshold measurements indicate that an increase in fiber excitability occurs during the period of increased conduction velocity. Somatic depolarization current delivered through the microelectrode will initiate spikes at the initial axon segment that show sustained firing in excess of 200 spikes per second. But, with repetitive high frequency antidromic stimulation the spikes fail to occur after an initial burst. In conclusion, the excitability and conduction velocity of a Cd efferent axon increases during the aftermath of a single action potential, and a region of low safety factor may exist along these fibers. This indicates that Cd axons may be operating not as simple relay lines, but as integrative elements responding in terms of their previous activity. (This work was supported by NIH Grants NS00405 and RR5384.)

119 PROJECTIONS FROM THE SUBSTANTIA NIGRA PARS RETICULARIS IN THE CAT USING A NEW STEREOTAXIC TECHNIQUE. K. Kultas-Ilinsky, I. Ilinsky*, L. C. Massopust, P. A. Young, and K. R. Smith. Depts. Anat. and Surg., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

The purpose of this investigation was to examine the topographical organization of the efferent projections from the substantia nigra pars reticularis (SNr) employing an extremely accurate stereotaxic technique. The technique used in this study is based on intracerebral coordinates and contrast ventriculography, allowing for the placement of injections of tritiated amino acids and radiofrequency lesions into small discrete parts of the SNr without encroaching on surrounding structures. The injections of tritiated leucine and proline were made in the anterior, intermediate or posterior parts of the SNr. Nigrostriatal fibers from all three parts coursed in a rostro-lateral direction, passed around the subthalamic nucleus without entering it, crossed the internal capsule and entered the entopeduncular nucleus, putamen and globus pallidus. Terminations in these structures were sparse and diffuse. Another bundle of fibers contained within the dorsomedial part of the internal capsule coursed dorsally and entered the posterior third of the caudate nucleus forming a rather dense terminal field. When injections of tritiated amino acids were confined to the anterior two thirds of the SNr, nigrothalamic projections were also labelled. These fibers, after leaving the SNr, coursed medially towards the midline, passed around the fasciculus retroflexus, and entered Forel's field where they continued anteriorly dividing into two groups. One turned sharply dorsally, passed around the centrum medianum and entered the ventromedial thalamic nucleus (VM) from its dorsoposterior aspect. The second group of fibers continued rostrodorsally a short distance in Forel's field to enter the VM from its ventral aspect. Very dense terminal accumulations of radioactive label were found throughout the VM from both of these projections. It was noted that when neurons exclusively in the most ventral part of the SNr were labelled, terminations were also apparent in the ventral anterior and ventral lateral thalamic nuclei. Verification of nigrothalamic terminations was obtained through electron microscopy. Radiofrequency lesions were made in the SNr resulting in degeneration of synaptic boutons and medium size myelinated axons within the VM. The degenerating boutons formed symmetrical synaptic contacts on the dendrites. In some cases they were found within glomeruli where they contacted several types of profiles. In conclusion, all parts of the SNr project to the striatum, with major terminations in the posterior part of the caudate, whereas only the anterior two thirds of the SNr projects to the thalamus where the main terminal area is in the VM. (Supported by USPHS FR 05388.)

120 EFFECTS OF BASAL GANGLIA STIMULATION ON BRAINSTEM TRIGEMINAL NEURONS. T. Labuszewski* and T. I. Lidsky. Dept. Psychol., SUNY at Stony Brook, N.Y. 11794.

Basal ganglia influences upon brainstem trigeminal sensory neurons were investigated. Units were recorded in and around the trigeminal principal sensory nucleus of chloralose anesthetized cats. Cells were classified as trigeminothalamic relay neurons, trigeminal non-relay neurons and reticular formation neurons on the basis of histological location and responsiveness to electrical stimulation of the posterior thalamus and natural stimulation of the face and mouth. Stimulation of the basal ganglia was applied via electrodes implanted bilaterally in the head of the caudate and in the entopeduncular nuclei. Stimulation of the basal ganglia evoked unit responses in trigeminal relay and non-relay cells, as well as reticular formation cells. Latencies typically ranged from 15-25 msec. The effect of basal ganglia stimulation upon reticular formation neurons tended to be more pronounced than the effect upon trigeminal nucleus neurons. Stimulation of the basal ganglia also evoked changes in trigeminal neuronal response to natural stimuli. A conditioning-test (C-T) procedure was employed in which an assessment was made of the effects of conditioning stimulation of the caudate upon test responses evoked in trigeminal neurons by stimulation of the sensory receptive field. Basal ganglia stimulation resulted in primarily inhibitory effects upon test responses at C-T intervals as long as 200 msec. Control procedures demonstrated that the results reported here cannot be ascribed to current spread to the internal capsule.

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121 ACETYLCHOLINESTERASE IN THE SUBSTANTIA NIGRA AND CAUDATE-PUTAMEN: PROPERTIES AND LOCALIZATION ON DOPAMINERGIC NEURONS AND TERMINALS. John Lehmann* and H.C. Fibiger (SPON: V.K. Singh). Division of Neurological Sciences, Univ. of British Columbia, Vancouver, B.C.

In order to selectively destroy neurons and terminals of the dopaminergic nigrostriatal tract, 6-hydroxydopamine (6OHDA) was administered to rats by one of two routes, intraventricular (IV) injection or unilateral stereotaxic injection into the nigro-striatal bundle (NSB). After a survival period of 4 to 5 weeks, enzymes in the 6OHDA-lesioned groups showed the following changes in activity (expressed as per cent of controls) in the substantia nigra (SN) and caudate-putamen (CP):

	Tyrosine hydroxylase	Choline acetylase	AChE
IV 6OHDA SN	10.3***	88.0	69.0***
IV 6OHDA CP	9.3***	93.6	87.6*
NSB 6OHDA SN	9.9***	99.6	56.7***
NSB 6OHDA CP	3.4***	106.0	87.9**

*p<.05; **p<.01; ***p<.001

To be certain that the observed decreases in cholinesterase activity were due to true acetylcholinesterase (AChE, EC 3.1.1.7) and not to butyrylcholinesterase (BChE, EC 3.1.1.8), several criteria were applied to tissues from the NSB-6OHDA group: 1) Substrate specificity. Acetylcholine (ACh) was replaced by either acetyl-β-methylcholine or butyrylcholine in the cholinesterase assay. SN and CP from 6OHDA lesioned rats showed 54% and 92% of control tissue cholinesterase activity respectively with acetyl-β-methylcholine as substrate, in good agreement with values found using ACh. No decrease in activity toward butyrylcholine was observed. 2) Kinetics. The decrease in cholinesterase activities at different concentrations of ACh was determined; the data showed that the 6OHDA-depleted cholinesterase was inhibited by high ACh concentrations. This is a characteristic property of AChE but not BChE. 3) Selective inhibitors. In the SN, the 6OHDA-depleted cholinesterase was inhibited by the selective AChE inhibitors BW284C51 and ambenonium with a dose-response similar to erythrocyte AChE but different from serum BChE. The selective BChE inhibitor, isoOMPA, inhibited the 6OHDA-depleted enzyme only at concentrations which inhibit erythrocyte AChE, concentrations somewhat higher than those which inhibit serum BChE. These results support the results obtained histochemically by Butcher et al. (J. Neural Transmission 37:127, 1975), and indicate that AChE is contained in dopaminergic neurons of the SN. Moreover, this is the first characterization of AChE from a homogeneous population of non-cholinergic neurons in mammalian central nervous system.

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122 PHARMACOLOGIC AUGMENTATION OF ACETYLCHOLINE LEVELS IN KAINATE-LESIONED RAT STRIATUM. Edythe D. London* and Joseph T. Coyle Depts. of Pharmacology and Psychiatry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA.

Stereotaxic injection of kainic acid (Ka) into the rat striatum produces neurochemical and histologic alterations in the nigro-striatal circuit similar to those seen in the neurodegenerative disorder, Huntington's Disease (HD). Ka injection causes degeneration of cholinergic and GABAergic neurons intrinsic to the striatum but leaves the nigral dopaminergic projection to the striatum intact (Coyle and Schwarcz, Nature 263:244, 1976). Since pharmacologic correction of the striatal acetylcholine (ACh) deficiency might ameliorate the dyskinesia of HD, we have examined the effects of systemic treatment with choline, dimethylaminoethanol (Deanol) and physostigmine on the levels of ACh in the striata of Ka-lesioned and control rats.

Rats received a stereotaxic injection of 3 μg of Ka in the left striatum. Four days after the lesion, the rats received the drugs IP injection and were killed by focused microwave irradiation at various times after treatment (30 min, physostigmine; 15 min, Deanol; 40 min, choline). The levels of endogenous choline, ACh and GABA were measured in Ka-lesioned, contralateral and

Treatment	Control	Contralateral	Ka-lesion
	ACh ± S.E.M. (p mol/mg tissue)		
Vehicle	56.1±3.2	51.5±2.2	14.9±0.9
Choline (100 mg/kg)	66.8±4.0	80.0±2.2	36.9±4.3
Deanol (408 mg/kg)	44.4±2.8	78.1±12.3	36.2±8.2
Physostigmine (1 mg/kg)	110. ±7.4	89.9±10.4	33.7±9.3

control striata. In Ka-lesioned striata, the levels of endogenous GABA and ACh were reduced to 46% and 27% of control respectively whereas the concentration of choline was unaffected. Choline treatment caused a 19% increase in ACh in control striata, a 55% increase in contralateral striata but a 120% increase in the lesioned striata. Although Deanol did not alter ACh in controls, it increased the levels by 52% and 114% in contralateral and lesioned striata respectively. Physostigmine caused a similar relative increase in control, contralateral and lesioned striatal ACh. These studies indicate that the deficiency in striatal ACh in the Ka-lesioned rat can be partially corrected by agents that increase precursor availability or inhibit acetylcholinesterase. Notably, choline and Deanol exhibit relatively greater effects in the Ka-lesioned striata than in controls; thus, the synthesis or storage of ACh in the surviving cholinergic neurons in the lesioned striatum may be altered. (Supported by USPHS Grants MH 26654, NS 13584, MH-RCDA, and National Foundation Grant to JTC and Fellowship MH-07142-01 to EDL).

123 COURSE OF NEOSTRIATAL EFFERENT FIBERS INVOLVED IN THE EXPRESSION OF ROTATIONAL BEHAVIOR IN RATS. John F. Marshall* and Urban Ungerstedt* (SPON: E.B. Goldstein). Karolinska Institutet, Stockholm, Sweden.

Rats given a unilateral injection of 6-hydroxydopamine along the ascending dopamine fibers of one hemisphere turn vigorously away from the side of the cerebral injection when administered apomorphine (0.25 mg/kg i.p.) 1-3 weeks later. This contralateral rotation appears due to the development of a supersensitivity to dopamine receptor stimulants in the denervated neostriatum.

Extensive electrocoagulations of the caudate-putamen performed in the same hemisphere as the prior 6-hydroxydopamine injection largely blocked the apomorphine-induced rotation. Electrocoagulations of the internal capsule during its telencephalic or diencephalic course were similarly effective in blocking this drug-induced behavior. However, extensive lesions of the globus pallidus which largely spared the internal capsule were without effect.

Knife cuts made in the coronal plane immediately rostral to the substantia nigra reduced the rotation by 54%. Knife cuts in the same plane immediately caudal to the nigra were less effective, blocking rotation by 25%.

These results suggest that neostriatal efferent fibers, in particular the strionigral projection, are involved in the expression of this dopamine-dependent behavior. (Supported in part by grant No. MH 27441 to J.F.M.)

124 CONTRAVERSIVE CIRCLING INDUCED IN THE RAT BY THE INTRANIGRAL MICROINJECTION OF MUSCIMOL. G. E. Martin, C. B. Bacino* and N. L. Papp.* Merck Institute, West Point, PA, USA, 19486.

GABA is thought to act within the substantia nigra (SN) to inhibit the firing of dopaminergic cells which project to the striatum. Accordingly, the direct unilateral application of a GABA agonist into the SN should result in the inhibition of firing of striatal dopamine neurons resulting in a preferential activation of striatal neurons on the side of the brain contralateral to the injection. It was anticipated that such treatment would evoke an ipsilateral turning response. We tested this hypothesis following the unilateral intranigral injection of muscimol, a potent GABA agonist.

A 24 ga stainless steel cannula was implanted stereotaxically in each of 38 rats at a site in the SN. As an anatomical control, microinjections were made within the caudate nucleus. To antagonize the turning evoked by muscimol, bicuculline (B) was administered i.p. or intranigrally. In addition, GABA in doses ranging from 2 to 50 μg was injected into the SN. All microinjections were delivered via a 30 ga cannula in a volume of 0.5 μl. Muscimol was administered in doses of 0.005, 0.05, 0.5 and 5.0 μg and a CSF vehicle control injection was made in each of 10 animals. The injections in each animal were spaced by an interval of one week. Turning was measured for 2-min intervals at 10 min before and at 5, 15, 30 and 60 min after the injection. At the end of the experiment, each microinjection site was verified histologically.

Muscimol, at the two highest doses, evoked a marked contralateral turning response significantly different from the response to artificial CSF. No ipsilateral turning was observed. The mean maximum turning response was observed within 30 min of the injection and reached 2.9, 2.5, 6.1 and 14.8 turns per minute (TPM) for the increasing doses of muscimol. B, administered i.p., did not diminish the turning evoked by muscimol, whereas the intranigral application of B only partially antagonized the turning response. Injected in the SN, B alone did not evoke a significant amount of turning. Neither the microinjection of GABA in the SN, nor the administration of muscimol in the striatum evoked any turning behavior.

At the doses tested, muscimol elicited turning contralateral to the injection site in the SN in agreement with the previous report of contralateral turning after the application of the putative GABA agonist, baclofen in the SN (Kelly and Moore, Pharmacologist, 1976, 18: 130). The mechanism of action of muscimol in inducing contralateral turning requires further investigation, especially in relation to the currently held views on the GABA-like action of this compound and the function of striato-nigral GABAergic neurons.

125 DESCENDING PROJECTIONS OF THE FELINE GLOBUS PALLIDUS.
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The external segment of the globus pallidus may influence the somatic motor system through projections to the subthalamic nucleus and substantia nigra. We have identified an additional projection which may also influence motor output.

As predicted from degeneration and autoradiographic studies, we found entopeduncular nucleus (feline homologue of primate internal segment of globus pallidus) neurons antidromically activated by stimulation in the region of the pedunculopontine nucleus. However, 8 of 105 neurons in the external segment of the globus pallidus were also antidromically activated. The latency of activation was 0.5 to 2.0 msec and the neurons were located in the caudal portion of the nucleus. Autoradiographic and horseradish peroxidase (HRP) experiments were performed to determine the terminal field and cells of origin of this descending pallidal projection. In autoradiographic experiments, 0.2 ul of ^{3}H leucine (100 uC/ul) was injected into the external segment of the globus pallidus. While a dense terminal zone was located in the subthalamic nucleus, some axons continued caudally to terminate in pars reticulata and pars compacta of the substantia nigra; some axons projected further caudally and dorsally to terminate in the caudal mesencephalon medial to the medial lemniscus, ventral to the inferior colliculus. This caudalmost terminal zone is primarily lateral to the entopeduncular nucleus projection in the pedunculopontine nucleus, but the two projections overlap slightly. In retrograde transport experiments 0.2 ul of a 50% solution of HRP (Type VI, Sigma) was injected into terminal regions of pallidal neurons. Following injections into medial substantia nigra, retrogradely labeled cells were located in the rostrolateral portions of the external pallidal segment. Injection into the lateral portions of the substantia nigra or lateral to the pedunculopontine nucleus resulted in labeled cells in the caudal ventral part of the nucleus. It appears that, in contrast to the entopeduncular nucleus, the descending projections of the external pallidal segment are topographically arranged.

126 TOXIC EFFECTS OF KAINIC ACID ON STRIATAL NEURONS: AGONISTS AND ANTAGONISTS.
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Local injections of kainic acid (KA) have been shown to destroy neurons with cell bodies in the injected area. The mechanism is not yet clear although it has been suggested that glutamate receptors may be the point of initial attach (1) and electron microscopic studies using small amounts (1-2.5 nm) of KA are consistent with this hypothesis (2). We have sought to establish what other agents might produce a similar effect and whether the KA action can be modified by concomitant injection of various drugs or prior pharmacological or surgical treatment. All injections were made into the caudate/putamen and choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD) activities in the striatum were used as indices of neuronal degeneration. Both the time and volume of injection must be kept constant to ensure reproducible results.

Significant losses in both enzymes followed stereotaxic injections of 40 nm DL-homocysteate or 50 nm L-glutamate but not after 10 nm ibotenic acid, muscazone, allylglycine or methionine sulfoximine. The effect of small amounts (2.5-5 nm) of KA were antagonized by coinjection of 50 nm of α-methylglutamate, diethyl kainate, ornithine, diaminopropionic acid, or diaminobutyric acid but not by 2-amino-4-phosphonobutyric acid, 2-amino-3-phosphonopropionic acid, 2-aminopropylphosphonic acid, proline or H966. They appeared to be increased in rats given 100 mg/kg of DOPA ip (together with an MAOI) and to be decreased in rats treated chronically with isoniazid under conditions reported to raise brain GABA levels. The effect of 2.5 nm of KA on cholinergic (but not GABAergic) neurons was significantly increased in animals where the nigrostriatal dopaminergic tract had previously been destroyed by 6-OHDA treatment. Other pretreatments tried included chronic morphine implantation, chronic reserpine, chronic haloperidol, Na di-n-propylacetate, γ-hydroxybutyric acid amantadine, aminooxyacetic acid, 5-HTP or taurine while coinjections were made with dopamine, atropine, arecoline or GABA.

(1) Olney et al, 1975 Neuroscience Meeting
(2) Hattori et al, Brain Res. in press, and this meeting

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127 CELL CLUSTERING IN EARLY AND LATE POSTNATAL MOUSE STRIATUM.
Patricia L. Mensah, Dept. Anat., Sch. Med., USC, Los Angeles, Ca. 90033.

Characteristics of cell clustering in the postnatal neostriatum were studied in mice from the day of birth to 6 weeks of age. Paraffin embedded material was sectioned at 10 microns in transverse, horizontal or sagittal planes and stained with either cresyl violet or with a modification of the Cajal on-the-slide silver staining procedure. Based upon earlier observations, the head of the caudate nucleus was divided into 3 zones - a central core region bordered by lateral and medial peripheral areas - and the body of the nucleus into dorsal and ventral zones. In adult mouse, the central core region of the head and ventral portion of the body contain most of the large (25-30 μ) cells of the structure. This study concentrated on studying the appearance of these areas at different postnatal times.

Neuronal cell clustering is a consistent feature of all zones at all postnatal times. Clusters range in size from a mean of 6 neurons per group at 3 days of age to a mean of 12 at 15 days or later. Even as early as day 0, only one large cell was present in the same cell grouping. Large fusiform cells occur most often in the central core area of the head or ventral area of the body of the nucleus after postnatal day 7. Cell clustering is extremely rigid in the central core zone of the head of the nucleus where both clumps and rings of clustered cells occur. Although present in other zones as well, the boundaries of individual groupings in these areas may be difficult to identify due to the tendency of some cells to occupy a position between neuronal groupings. This tendency of cells to occupy areas outside of any clearly defined cell aggregation is a developmental phenomenon that increases with advancing age.

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128 POSTSYNAPTIC POTENTIALS EVOKED IN DEVELOPING CAUDATE NUCLEUS NEURONS BY ACTIVATION OF THEIR AFFERENTS.
R. Morris,* E Cherubini* C.D. Hull, M.S. Levine and N.A. Buchwald. MRRC, Sch. of Med., UCLA Los Angeles, CA. 90024.

Synaptogenesis in the caudate nuclei of cats starts prior to birth and extends into the postnatal period. By thirty days (oldest age examined) adult synaptic densities have not been reached (Adinolfi, A. Brain Res. in press). To extend our extracellular single unit studies of the postnatal period of synaptogenesis we are currently carrying out an intracellular study of developing caudate neurons.

Kittens (2-72 days of age) were anesthetized with NO_2 (60-70%) O_2 (30-40%) and Halothane (1-0.5%). Stimulating electrodes were placed stereotaxically in the ventral midbrain, medial posterior thalamus (intralaminar nuclei region) and on the surface of the precruciate cortex. The cortex overlying the head of the caudate nucleus was ablated allowing direct visual placement of recording electrodes.

In animals under 15 days of age (4 animals, 10 neurons) prominent excitatory postsynaptic potentials (EPSPs) (4-10 mv) were evoked in caudate neurons by stimulation (10-15 v, 0.1-1.0 msec) of all three regions. At low frequencies of stimulation (.5-1 Hz) these EPSPs usually depolarized the cell sufficiently to result in spike initiation, however, higher rates of stimulation (above 8 Hz) the EPSPs continued to be evoked by each stimulus but few resulted in spike initiation. In four of these units the initial depolarization was followed by a period of hyperpolarization (3-6 mv, 70-90 msec duration). This is in clear contrast to responses observed in adults and older kittens (above 50 days) where EPSPs particularly those evoked by cortical stimulation are followed usually by prominent long duration hyperpolarizations (Inhibitory Postsynaptic Potentials, IPSPs). In animals 15-30 days of age (8 animals, 40 neurons) 17 of the 29 units responding to cortical stimulation showed initially an EPSP followed by a period of hyperpolarization whose magnitude and duration was closer to that of the IPSPs found in adult neurons. Typically, however, these IPSPs also tended to be smaller in amplitude and shorter in duration than those found in mature animals. Repetitive stimulation at frequencies above 20 Hz produced prolonged depolarization during stimulation, again indicating the less potent nature of the IPSPs. We have examined three animals above 45 days old (24 neurons) and here IPSPs of comparable magnitude and duration to those found in adults were observed.

These results are in agreement with our earlier extracellular data indicating the rapid postnatal development of excitatory responses in caudate neurons. In addition the current results suggest that the inhibitory processes show a slower more gradual rate of development than is true for the excitatory processes.

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129 NIGRAL DESCENDING EFFECTS ON CERVICAL AFFERENT TERMINAL EXCITABILITY AND DORSAL ROOT POTENTIALS. Fereshteh Motamedi* and Donald H. York, Dept. of Physiol., Sch. of Med. and Dalton Res. Center, Univ. of Missouri, Columbia, Mo. 65201

Previous behavioral studies have demonstrated head turning movements upon stimulation of the basal ganglia. The neural pathways mediating this behavior have not been defined. Therefore the present study was undertaken to study the effects of stimulation of the substantia nigra on changes in exciteability of neck muscle afferents at the level of the cervical spinal cord. Twelve adult male Sprague-Dawley rats (500-600 gm) were anesthetized with urethane and a cervical laminectomy was performed. Dorsal root potentials (DRP) were recorded from C_1 or C_2 dorsal roots with platinum bipolar electrodes and evoked by stimulation in the dorsal horn of the cervical spinal cord. A bipolar stimulating electrode was placed in the substantia nigra (pars reticulata), and was subsequently histologically verified. A train of conditioning pulses delivered to the substantia nigra (pars reticulata) (25-60 μA, 0.1 msec, 500/sec, 20 msec) evoked a DRP. A decline in a spinal evoked DRP was observed when a nigral train of pulses preceded the spinal evoked DRP by 5-10 msec. When the stimulating electrode was confined to substantia nigra (pars compacta), no decrease in spinal evoked DRP was observed. These results indicate that there may be a presynaptic facilitation of cervical afferent terminals by a descending nigral pathway. This suggests that such a pathway may contribute to the mechanism responsible for head turning movements during stimulation of the basal ganglia.

130 THE USE OF KAINIC ACID IN THE ANATOMICAL LOCALIZATION OF ENZYMES IN THE SUBSTANTIA NIGRA. J. Nagy*, S. R. Vincent*, J. Lehmann*, E. G. McGeer and H. C. Fibiger (SPON: S. C. Sung). Div. of Neurol. Sci., Univ. of British Columbia, Vancouver, B. C. V6T 1W5.

Kainic acid (KA) was stereotaxically injected into the substantia nigra (SN) to gain further information as to the localization of the enzymes choline acetyltransferase (CAT), glutamic acid decarboxylase (GAD), acetylcholinesterase (AChE), and dopamine-sensitive adenylate cyclase (DAC) within this nucleus. These studies were made possible by previous findings which suggest that KA, when injected intracranially in a variety of brain nuclei, destroys all neuronal cell bodies, while producing minimal damage to axonal terminals in the area of injection.

A unilateral injection of 5 nmoles of KA in a volume of 1 μl into the SN resulted 2 weeks later in virtually complete neuronal degeneration in this structure as demonstrated by histological examination. Also tyrosine hydroxylase activity (TH) in the ipsilateral caudate nucleus was almost completely depleted. The enzyme activities in the table are presented as percent of control, both per mg protein and per SN. This is desirable since the

Enzyme Activities Following Intranigral Kainic Acid

Enzyme	Per mg Protein	Per SN
TH (caudate)	6.1 ***	--
GAD (caudate)	133.8	--
GAD	51.1 ***	41.4 ***
CAT	124.5	79.0
AChE	56.0 ***	36.1 ***
DAC basal	151.2	110.8
1 μM Dopamine	146.1 *	126.8
10 μM Dopamine	172.9 *	124.8
100 μM Dopamine	166.2 **	122.2
NaF	135.5	87.3

(* $p<.05$; ** $p<.02$; *** $p<.001$; Student's t, two-tailed)

shrinkage of the SN may lead to spurious results if the enzyme activities were expressed only on the per protein basis. The persistence of DAC in the face of almost total neuronal cell loss supports the finding that DAC is not present on dopaminergic soma in the SN and establishes that this enzyme is located exclusively on nerve terminals. Similarily, the failure of KA to affect CAT activity suggests that the source of CAT is external to the SN. The partial decrease in AChE suggests both a cellular and terminal localization of this enzyme. Although unlikely, a glial contribution to these enzyme activities cannot be excluded. Finally, although an effect of DA on terminals cannot be ruled out, the partial decrease in GAD activity observed suggests that some of the GAD is contained in interneurons within the SN.

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131 REGIONAL DISTRIBUTION OF DOPAMINE METABOLITES IN THE CAUDATE OF RAT AND RHESUS MONKEY. N. Narasimhachari, R.C. Smith*, T. Samorajski* and J.M. Davis (SPON: G. Pandey). Illinois State Psychiatric Institute, Department of Research, Chicago, Illinois 60612. *Texas Research Institute of Mental Sciences, Houston.

In our studies on the acid metabolites of dopamine in the caudate of rats we noticed wide variations in HVA levels depending on the size of the caudate dissected. Small samples (10-50 mg) gave values ranging from 3 μg/gm to 15 μg/gm (SEM 2.91 μg/g) and larger samples (80-140 mg) gave values from 1 to 1.5 μg/g (SEM 0.09 μg/g). This suggested differences in the regional distribution of dopamine and its metabolites in the caudate. We have now investigated HVA and 3-methoxytyramine levels in discrete areas of the caudate of rat and rhesus monkey. The amine and acid metabolite levels were determined in the right and left caudate of the rat, the tissue was also dissected to three parts, (anterior, midsection and posterior) weighing approximately 10, 20, and 10 mg respectively. Similarly eight sections each were dissected out from the right and left caudate tissue of a rhesus monkey. HVA was derivatized to methyl ester-TMS ether (1) and 3-methoxytyramine to isothocyanate-TMS ether (2) and quantitated by mass fragmentography. Significant differences in the regional distribution of HVA levels were noticed in the rat caudate and in the monkey. In the monkey the values ranged from non-detectable to 4 μg/g. Zimmerberg et al (3) reported significant asymmetry in striatal dopamine content. Further work on the neuroanatomical differences underlying the differences in dopamine metabolites in discrete areas of the two sides of the caudate is in progress.

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132 MOTOR CORTEX PROJECTIONS TO THE CAUDATE NUCLEUS. II. NEUROANATOMY. A. Nieto,* E. Garcia-Rill and A.M. Adinolfi. MRRC, Sch. of Med., UCLA, Los Angeles, CA. 90024.

This study describes the distribution of fibers from the precruciate motor cortex to the head of the caudate nucleus. Discrete lesions were made by suction ablation in lateral areas (forelimb representation) and in medial areas (trunk representation). Care was taken not to involve the underlying white matter. The cats were sacrificed one week later. Serial coronal and sagittal sections, stained by the method of Fink and Heimer, were analyzed to determine the patterns of terminal degeneration. Small unilateral lesions of the medial precruciate cortex produced diffuse terminal degeneration within the lateral one-half of the rostral head of the caudate nucleus on both sides. Lateral precruciate lesions, involving only the forelimb areas on one side, resulted in bilateral terminal degeneration. However, its distribution to rostral caudate nucleus was confined to a circumscribed area immediately adjacent to the internal capsule and within the projection area of the medial motor cortex. In all animals, the medial precruciate lesions resulted in more terminal degeneration than the lateral lesions and the degeneration is always more pronounced ipsilaterally. In more caudal sections, dense terminal degeneration was found also in the nucleus accumbens.

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133 HYPERACTIVITY VERSUS HYPERREACTIVITY IN FRONTAL CORTEX AND CAUDATE LESIONED CATS. Ch.E. Olmstead and J.R. Villablanca, Dept. Psychiat., Ment. Retard. Res., Ctr., UCLA, CA, 90024.

An open field room (12 ft X 12 ft) was used to evaluate and compare the behavior of normal male (INT, N=5) and oestrous female (OE, N=4) cats and animals receiving ablations of the: a) caudate nuclei (BAc, N=4), b) frontal cortices (BFr, N=5), c) one cerebral hemisphere (large lesion control) (HEMI, N=5). All lesions were performed in adult animals which were tested for three 15-min sessions at approximately one week intervals. Following the completion of the three-day baseline periods various stimuli were introduced into the unenriched environment and changes in the activity of the animals were assessed in 3-5 additional sessions. The basic data were the number of 27 in^2 squares crossed in one minute intervals. The INT were undistinguishable from HEMI animals and both groups showed decreased activity across the minutes within individual sessions ($p < .001$) and between individual days ($p < .001$) of testing. Their adaptation (habituation) to the test room was essentially complete by the end of the second testing day. Only the tendency of the HEMI animals to ipsilateral rotation distinguished them from the intact males. The BAc, on the other hand, showed less decrease of activity as a function of time both within ($p < .001$) and between sessions ($p < .006$) in the bare open field and were actually most active on the second habituation day. In contrast, BFr and OE animals were extremely active and showed increased activity as a function of days ($p < .01$). Within a session both of the latter groups showed variability far in excess of that observed in the other three groups. The introduction of olfactory, visual, auditory and somatosensory stimuli into the field had the most profound, albeit different, effects on the BAc and BFr groups. The INT, HEMI and OE cats made only cursory inspections of the stimuli and then proceeded to go to sleep. BFr cats showed an initial freezing to the onset of the stimulus followed by a somewhat sporadic approach. BAc animals, regardless of lesion size, spent the longest time inspecting the new stimuli and, irrespective of the modality, appeared to show a preference for the first objects or stimuli presented. Therefore, where BAc animals showed predominantly increases in reactivity, the animals with frontal lesions and those in active oestrous showed generalized activity increases both in the empty and in the stimulus rich open field. These data suggest that: a) the stimulus properties of the environment and their subsequent modification via conditioning might explain some of the behavioral observations previously made in acaudate cats; b) that other dynamic components, rather than just simple habituation, are operating in the adaptation of the individual groups to the open field. (Supported by USPHS Grants HD-05958, MH-07097 and HD-94612).

134 RESPONSES OF CAUDATE NUCLEUS NEURONS TO INTRACELLULAR STIMULI. R.J. Preston*, M. Sugimori* and S.T. Kitai. Morin Memorial Lab., Dept. Anat., Sch. Med., Wayne State Univ., Detroit, Mich. 48201.

Neurons in the cat caudate nucleus (Cd) which were monosynaptically excited by substantia nigra (SN) or thalamic (CM) stimulation were also analyzed for responses to direct transmembrane stimuli. Direct stimuli were applied through glass electrodes (1.5M KCl; 30-50 MΩ) which simultaneously registered intracellular potentials. Animals were anesthetized with pentobarbital, artificially respirated and paralyzed with flaxedil. The parameters of constant current, rectangular direct stimuli were adjusted to obtain data on passive electrical constants as well as spike discharge properties of the impaled Cd neurons.

Input resistance (R_O) (12 to 24 MΩ, $\bar{X}$=16.5, N=16) was determined from plateau values of membrane responses to hyper- and depolarizing stimuli. Membrane time constants were estimated from the exponential time course of responses to weak current pulses (±0.5 nA) and ranged from 7.8 to 16.9 msec (mean 11.3 msec). Since the recorded cells are probably medium spiny neurons, membrane resistance for an average cell is calculated at nearly 8000Ω·cm^2. A resistance of this magnitude would ensure efficient electrotonic spread of synaptic currents from distal dendrites to an axon trigger zone.

Single or repetitive spike discharge in Cd neurons was readily evoked by SN or CM stimuli or by suprathreshold direct depolarizing stimuli. Spike threshold was higher for direct than for synaptic activation (mean 7.2 vs 5.6 mV) and while firing frequencies were similar for either stimulus mode (100-200 spikes/second) direct stimuli were able to elicit sustained spiking with little adaptation. Neurons that showed spike properties suggesting accommodative regulation of firing threshold (e.g., short latency with rheobasic stimuli; low capacity for sustained firing) were those that appeared damaged by impalement (spike potentials less than 45 mV; membrane time constants less than 5 msec). These data suggest that the frequently noted silence of Cd neurons is more likely due to inhibitory synaptic influence than to intrinsic membrane specialization.
(This work was supported by NIH Grant NS 00405 and RR 5384.)

135 NEUROTRANSMITTER RECEPTOR ALTERATIONS IN PARKINSON'S DISEASE. T.D. Reisine*, J.Z.Fields*, E.D.Bird*, P.Schreiner*, H.I.Yamamura and S.J.Enna. Univ. of Ariz. Health Sci. Ctr., Tuc. AZ 85724; Addenbrooke Hosp., Cambridge, Eng.; Univ. of Texas, Houston, TX 77025.

Investigation of neurotransmitter levels, and related enzyme activities in human postmortem brains have helped elucidate the pathophysiology of a number of neurological diseases. Parkinson's disease (PD) is a chronic and progressive degenerative disease characterized by lesions in the dopamine (DA) containing neurons in the zona compacta of the substantia nigra. The ability of L-dopa to alleviate the symptoms of PD is thought to derive in part from a supersensitivity of the denervated DA receptors in the basal ganglia (BG). In this study we examined the BG and frontal cortex of PD and control brain samples for alterations in receptor binding using ^{3}H-spiroperidol, ^{3}H-GABA, ^{3}H-QNB and ^{3}H-5HT, respectively. Eighteen postmortem brain samples, nine with PD and nine controls were assayed for receptor alterations as well as for choline acetyltransferase (ChAc) activity.

In the putamen of PD brains we found significant reductions in ^{3}H-5HT binding (36%), ChAc activity (55%), and significant increases in ^{3}H-QNB (30%) binding. In the caudate nucleus (CN), we found significant decreases of ^{3}H-spiroperidol (30%) binding in PD samples whereas ChAc activity remained unchanged. No significant receptor alterations were found in the globus pallidus although, there was a significant lowering of ChAc activity. ^{3}H-GABA binding remained unchanged in all brain areas examined.

Our findings of the positive correlation of ^{3}H-5HT binding with ChAc activity in the putamen of PD samples may indicate that 5HT receptors might reside on cholinergic interneurons within this area. The increase in ^{3}H-QNB binding within the putamen may be a manifestation of supersensitivity of muscarinic receptors resulting from the progressive loss of the striatal cholinergic interneurons.

The affinity of ^{3}H-spiroperidol binding as determined by Scatchard analysis in control brain is unchanged in PD. Thus the decrease of ^{3}H-spiroperidol binding in the CN appears to be due to a decrease in the density of DA receptors.

After long-term treatment of PD, some patients become refractory to L-dopa therapy. The results of our studies suggest that the lack of responsiveness to L-dopa therapy may result in part from a progressive loss of striatal DA receptors. The lack of correlation between DA receptor binding and ChAc activity in the CN suggests that either DA receptors are desensitized from the chronic L-dopa treatment or that some of the DA receptors are located on non-cholinergic neurons that have degenerated. Supported by Huntington Chorea Foundation and the Committee to Combat Huntington's Disease, NIH grants and RSDA.

136 AN AUTORADIOGRAPHIC ANALYSIS OF THE EFFERENT PROJECTIONS OF THE SUBTHALAMIC REGION IN THE RAT. Juarez A. Ricardo* (SPON: Walle J.H. Nauta). Dept. Psychol., MIT, Cambridge, MA 02139.

The subthalamic region seems to be innervated by several central structures, such as the cerebral cortex, the cerebellum, and various somatosensory nuclei. The study of its efferent projections, however, has been hampered in the past by the presence of a great number of fibers traversing the region. We decided to investigate this problem with the autoradiographic tracer technique, which is thought to be able to circumvent the fibers-of-passage difficulty. Small microelectrophoretic injections of tritiated proline and leucine in the zona incerta and in the nuclei of the fields of Forel in the rat revealed the existence of widespread descending and ascending projections which are mainly ipsilateral with minor crossed components. Fibers radiate dorsomedially from the injection site and lead to labeled fields in the thalamic parafascicular nucleus, extensive regions of the central gray substance (extending as far caudal as the genu of the facial nerve), interstitial nucleus of Cajal, and several of the pretectal nuclei (anterior, posterior and medial nuclei, and nuclei of the posterior commissure). Another component distributes itself to middle and deep layers of the superior colliculus, mainly in their lateral portions. A more directly descending component innervates the red nucleus (mainly its parvocellular portion) and a wide region of the midbrain reticular formation. This bundle of fibers continues caudalward, occupying a ventromedial position in the tegmentum, and at pontine levels innervates the nucleus reticularis tegmenti pontis, restricted sectors of the pontine nuclei, and the nuclei reticularis pontis oralis and caudalis; sparser labeling marks also the parabrachial region. At still more caudal levels this bundle comes to innervate the medial portions of the nucleus reticularis gigantocellularis and of the nucleus reticularis medullae oblongatae, pars ventralis, and restricted districts of the inferior olive; a smaller component of this descending bundle reaches the spinal cord. Ascending fibers lead from the injection site to labeled fields in several thalamic nuclei (ventromedial nucleus, nucleus reuniens, nucleus centralis medialis, nucleus paracentralis and nucleus centralis lateralis), in the entopeduncular nucleus, in the posterior hypothalamic nucleus, and in the lateral hypothalamic area. Preliminary evidence suggests that the subthalamic nucleus of Luys, on the other hand, projects significantly only to the substantia nigra (chiefly to its pars reticulata), to the entopeduncular nucleus, and to the external segment of the globus pallidus. (Supported by PHS Grant NS06542, by NSF Grant 7681227, and by FAPESP Grant 04-75/0167.)

137 AFFERENTS TO THE CAUDATE NUCLEUS IN THE CAT. G. James Royce. Dept. Anatomy, University of Wisconsin, Madison, WI 53706.

The horseradish peroxidase tracing method has been used to analyze the afferent connections of the caudate nucleus in the cat. Following HRP injections which were restricted to the head and body of the caudate nucleus, labeled neurons were present bilaterally throughout an extensive rostral zone of the neocortex. The most heavily labeled region of cortex was the anterior sigmoidal gyrus. Other cortical regions, which contained HRP labeled cells included the posterior sigmoidal, the anterior lateral, anterior suprasylvian, the anterior ectosylvian and the anterior sylvian gyri. Labeled cells were also apparent within the gyrus proreus, the anterior limbic cortex and the cingulate gyrus. All HRP positive cortical neurons were small to medium sized pyramidal cells. Such cells were located in layers II-VI, but were most prominent in layers III-V.

In addition to the prominent cortical regions of labeling, several thalamic nuclei also contained HRP-labeled neurons. The greatest number of labeled cells within the thalamus were located within the centromedian, the central lateral and the ventral anterior nuclei. Other thalamic zones including the central medial, the paracentral, the parafascicular, the dorsomedian, the lateral dorsal and the lateral posterior nuclei also contained labeled neurons.

Of particular interest was the presence of a significant number of labeled cells within a specific portion of the amygdaloid complex, i.e., the pars magnocellularis of the basal amygdaloid nucleus.

Finally, a large number of neurons within the pars compacta of the ipsilateral substantia nigra were densely labeled. In addition to this prominent ipsilateral projection, several scattered labeled neurons were also present within the ipsilateral pars reticularis and the contralateral pars compacta of the substantia nigra. (Supported by University of Wisconsin Graduate School Grant 135-4424).

138 IMPAIRED LEARNING AND MEMORY AFTER KAINIC ACID LESIONS OF THE STRIATUM: A BEHAVIORAL MODEL OF HUNTINGTON'S DISEASE Paul R. Sanberg, John Lehmann* and Hans C. Fibiger. Div. Neurol. Sci., Dept. Psychiat., Univ. of B.C., Vancouver, B.C., V6T 1W5

Intrastriatal micro-injection of kainic acid in rats has recently been shown to cause dramatic and permanent decreases in choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD), enzymes thought to be associated with cell bodies in the caudate-putamen (CP). In contrast, tyrosine hydroxylase (TH), an enzyme found only in nerve terminals whose perikarya are extrinsic to the CP is not affected. The selectivity of kainic acid in lesioning cell bodies but not terminals in the CP and its resulting biochemical sequelae have demonstrated that this procedure may be potentially useful as a biochemical model of Huntington's disease (HD) (Coyle and Schwarcz, *Nature 263*: 244, 1976; McGeer and McGeer, *Ibid*, 517). Clinically, HD is almost always associated with progressive intellectual deterioration and other psychological symptoms including impairments of memory and judgement. In this study the effects of kainic acid induced lesions of the CP on locomotor activity and on passive avoidance learning and memory were evaluated.

Bilateral injections of kainic acid (6 nmoles in 1 µl) into the CP of rats (n=11) resulted in impairment of both acquisition ($P < .001$) and 24 hr retention ($P < .01$) of a step-down passive avoidance task when compared to controls (n=12). Spontaneous locomotor activity measured in photocell activity cages was not significantly affected. Biochemical analysis on CP and cortical samples on behavioral subjects showed a significant reduction in CAT (22%) and GAD (25%) in the CP whereas TH activity was not significantly changed. The activities of CAT and GAD in cortical samples did not differ from controls, suggesting that the damage was confined to the CP. CP tissue weights were significantly less than controls (22%) which is consistent with previous findings in HD (Lange *et al*, *J. Neurol. Sci. 28*: 401, 1976). This decrease in tissue weight reflects shrinkage of the CP over the long survival period (75 days) and accounts for the smaller decreases in CAT and GAD reported here, compared to losses reported for shorter survival periods (McGeer and McGeer, *Nature 263*: 244, 1976; unpublished observations).

The present observation, showing that rats with these lesions also displayed marked deficits in learning and memory, indicates that this preparation may not only represent a biochemical model for HD but is a potentially useful animal preparation for studying the psychological deficits associated with this disease. Finally, because of the specificity of these lesions, it also suggests that the corpus striatum serves as an important neuronal substrate for complex psychological processes such as learning and memory.

Supported by the Medical Research Council of Canada.

139 SHORT-STEP LOCOMOTION RELEASED BY ANTI-CHOLINERGIC DRUGS IN THE AKINETIC, 6-HYDROXYDOPAMINE-TREATED RAT. Timothy Schallert*, Ian Whishaw*, V. D. Ramirez*, and Philip Teitelbaum. Dept. Psych., Univ. Illinois, Champaign, IL 61820.

Intraventricularly applied 6-hydroxydopamine (6-OHDA) severely depletes brain catecholamines in rats, and produces a complex set of symptoms that include akinesia or bradykinesia, and rigidity. Parkinson patients display similar disorders of movement. In both the akinetic rat and the Parkinson patient, the symptoms are thought to be related primarily to the disruption of dopamine transmission in the nigrostriatal system.

In the present study, using as a model the rat depleted of brain catecholamines by intraventricular application of 6-OHDA, we demonstrated that short-step locomotion, a form of walking strikingly analogous to the gait of Parkinson patients, can be temporarily released by anti-cholinergic drugs (atropine or scopolamine) in otherwise profoundly akinetic rats.

A radioenzymatic assay for dopamine and noradrenaline was used to confirm the effectiveness of the 6-OHDA treatment. The amount of walking was determined primarily by recording the number of revolutions in an activity wheel. Relative to control animals, the 6-OHDA-treated, catecholamine-depleted rats displayed a dose-dependent hyperkinesia when given atropine or scopolamine. The peripherally-acting anti-cholinergic drug, atropine methyl nitrate, had no significant effect.

The most profoundly akinetic rats showed the greatest amount of walking after anti-cholinergic drugs. For example, a rat that had never been observed to walk spontaneously showed nearly 3,000 activity-wheel revolutions in the eight hours following a single injection of 50 mg/kg atropine.

There were a number of interesting features in the locomotor behavior of the otherwise akinetic 6-OHDA-treated rats given atropine. For example, the walking steps released in the 6-OHDA rats were shorter than those of control rats given anti-cholinergics. To demonstrate this, we brushed the hind feet of the animals with ink and measured the distance between each of the resulting foot prints. The short-step, Parkinson-like gait of the 6-OHDA-treated animals was always quite striking.

In conclusion, our results support the long-established clinical value of anti-cholinergics in the treatment of some symptoms of human Parkinsonism, and suggest possible use to counteract akinesia. They also support the view that there is a mutually antagonistic interaction between catecholaminergic and cholinergic systems in the brain.

140 PROTEIN SYNTHESIS AND GLUTAMATE UPTAKE AND BINDING IN THE RAT NEOSTRIATUM FOLLOWING KAINIC ACID INJECTIONS. V. K. Singh, E. G. McGeer and P. L. McGeer. Kinsmen Lab. of Neurol. Res., Dept. of Psych., Univ. of British Columbia, B.C., V6T 1W5, Canada.

Stereotaxic injections of 1-5 nm of kainic acid (KA) into the caudate/putamen (CP) of a rat causes degeneration of neuronal cell soma in the injected area with concomitant losses in glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT); dopaminergic nerve endings are not destroyed and tyrosine hydroxylase (TH) activity is not reduced. These enzymic changes are comparable to those seen in Huntington's chorea. Changes in striatal proteins have also been reported in chorea. The incorporation of ^{3}H-leucine into proteins was examined at various times after the stereotaxic injections of 5 nm of KA into the CP; rats were killed 2 hours after the stereotaxic injection of ^{3}H-leucine and the amount of label in acid-insoluble material determined in the whole homogenates and in the nuclear and cytosol fractions. There was a 40-50% reduction in the total incorporation of ^{3}H-leucine at 1-2 days after the KA injection. At 3 days, however, the incorporation was increased to about 160% of control and was maintained at elevated levels until 5-10 days following the KA lesion. The reduction in incorporation at 1-2 days was limited to the cytosol fraction, whereas the increased accumulation at 3-10 days was in the nuclear pellet.

The binding of ^{3}H-colchicine to the cytoplasmic supernatant was decreased 30% and 60%, respectively, at 1 and 5 days after KA injections suggesting the loss of microtubular filaments of the neurons at the injection site.

Binding of ^{14}C-glutamate to the crude synaptosomal membrane fractions was reduced by 40-60% at 5 days after KA injections; this reduction is consistent with the existence of glutamate receptors on the affected neurons. High affinity, sodium-dependent uptake of glutamate into a synaptosomal preparation was not reduced in the CP of rats receiving 2.5 nm of KA and was increased significantly (to 137%) at a time (2 days) when GABA uptake was decreased to 57% of control, GAD to 64% of control and CAT to 62% of control. Since there is evidence that much of the high affinity uptake of glutamate in the striatum is into terminals of the cortico-striatal tract, this result is another indication that KA does not lesion primarily axon terminals. The increased uptake, like the increase in TH observed in KA-injected striata, suggests that the activity in the presynaptic boutons may be increased by either a presynaptic effect of KA itself or as a secondary response to degeneration of the post-synaptic elements.

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141 MOTOR AND BEHAVIORAL CHANGES IN CATS FOLLOWING CHOLINERGIC STIMULATION OF THE CAUDATE AND ENTOPEDUNCULAR NUCLEI. Paulette Springer* and Russell E. Dill. Dept. Anat., Baylor Coll. Dent., Dallas, Tx. 75246.

An electromyographic (EMG) study using fine wire electrodes was made of tonic activity of neck extensor muscles in cats following injection of carbachol (0.5-2 µg) into the head of the caudate nucleus and entopeduncular nucleus. Intracranial injections were made unilaterally via chronic cannulae implanted bilaterally.

Changes in muscle tone were recorded and quantitated by means of standard EMG equipment and spike counters with digital-to-analog converters. One second histograms were made of motor unit activity both ipsilateral and contralateral to the drug injection site prior to and at 10-20 min after drug injection. Similar records were made of saline injections in the same sites and compared with records obtained during carbachol treatment. All cannula sites were verified histologically.

Injection of carbachol in the caudate produced an eight fold increase ($P < 0.001$) in motor unit activity on the contralateral side and a three fold increase ($P < 0.001$) on the ipsilateral side as compared with saline effects. Carbachol injected into the entopeduncular nucleus produced a 560 fold increase ($P < 0.001$) in motor unit activity contralaterally and a 2.3 fold increase ipsilaterally. These EMG records verify the behavioral changes seen after injection of carbachol into the caudate and entopeduncular nuclei. These were circling and neck torsion respectively.

Other injection sites (internal capsule, cortex and nucleus accumbens septi) did not produce these effects.

142 DENDRITIC INHIBITION OF THE CAUDATE SPINY NEURON. C. P. VanderMaelen*, J. D. Kocsis* and S. T. Kitai (SPON: J. A. Rafols). Morin Memorial Lab., Dept. Anat., Sch. Med., and Dept. Psychol., Wayne State Univ., Detroit, MI 48201.

Intracellular recordings were made in the caudate (Cd) nucleus in cats anesthetized with α-chlorolose (80 mg/kg). Glass microelectrodes were filled with 2 M KCl and had DC resistances of 30 to 50 MΩ. In some instances the electrode was filled with 4% horseradish peroxidase (HRP) in 0.2 M KCl in order to intracellularly stain the recorded neuron. All identified neurons were medium sized, with aspiny somata and proximal dendrites, and spine laden distal dendrites. Stimulating electrodes were placed in the substantia nigra (SN) and the centro-median-parafascicular complex (CMP) of the thalamus. Stimulation of SN induced a short latency monosynaptic EPSP of low amplitude, designated C1; and a longer latency monosynaptic EPSP of large amplitude, designated C2. The C2 EPSP arose from the falling phase of the C1 EPSP. CMP stimulation always resulted in a slow monosynaptic, large amplitude EPSP similar to C2, but occasionally a C1-like response was also observed. Double stimualtion of SN or CMP resulted in amplitude reduction or elimination of the test C2 EPSP, while C1 remained unchanged. The reduction in the test C2 EPSP followed an orderly time course, beginning at conditioning-test interstimulus intervals (ISIs) of about 12 msec, and lasting up to 300 msec. With ISIs less than 12 msec, summation of C2 EPSPs occurred. In some cases IPSPs were observed following the C2 EPSP. The time course of the C2 reduction followed that of the IPSP. But even when no IPSP was seen, C2 reduction still occurred. In field potential analysis, SN stimulation evoked a negative-positive potential in the Cd nucleus. The positivity corresponded in time with the C2 EPSPs. Double stimualtion of SN resulted in a reduction of the positive field potential, with a time course paralleling that for the C2 EPSP reduction. These data indicate that in the Cd spiny neuron inhibition may be operating distally on the dendrites, "shunting" the C2 EPSP which is induced in the distal dendrites and the spines. The C1 EPSP may be generated on the proximal dendrites and/or on the soma, and is virtually unaffected by the dendritic inhibition. (This work was supported by NIH Grants NS00405 and RR5384.)

143 CATS WITH LESIONS OF THE CAUDATE NUCLEI ARE BEHAVIORALLY HYPER-REACTIVE. J.R. Villablanca and Ch.E. Olmstead, Dept. Psychiat., Ment. Retard. Res. Ctr., UCLA, CA, 90024.

We have previously demonstrated that hyper-reactivity to external stimulation is a component of the syndrome observed in adult cats receiving caudate nuclei ablations as kittens. Behavioral responses of 16 adult cats with ablations of the caudate nuclei through a midline approach (BAc, N=4), removal of the frontal cortices (BFr, N=5), and of intact cats (INT, N=7) were scored on a 6 point scale during presentation of recorded cat vocalizations or of pure tones. Twenty seven sequential 15 sec presentations of cat vocalizations were made at 1 min intervals on 2 consecutive days. One week later the animals were similarly tested using 2 kHz tone stimulus. All animals showed initial distinct behavioral responses to both vocalizations and tones. On day 1 of call presentations all INT animals, 3 BAc and 1 BFr showed the maximal response. During the first day's presentation INT animals showed the most rapid decrement followed by BFr and BAc cats. Where the BFr were readily distinguishable from BAc animals on day 1, on day 2 of call presentations they both showed very little savings from previous days and habituated similarly. On the first day of tone presentation INT animals showed savings from the previous habituation experience. BFr showed more savings than INT and BAc showed the least such that both lesion groups were not significantly different from day 1 of the cat call. Two additional differences were noted. First, when the INT stopped responding, no further responses were seen throughout the session, while the BFr showed periods of non-responses followed by brief bouts of responding and the magnitude of responding was in itself variable. Secondly, the response pattern of BAc animals fit well with the previously observed stereotyped behavior in BAc cats; i.e., the animals responded at the same intensity for long periods of time (i.e., runs of simple head turns). The levels of habituation described for both BAc and BFr cats remained stationary across several months. To better analyze behavioral responsiveness, some animals were tested using "conspecific" vocalizations and artificial laboratory sounds. This demonstrated that behavioral reactivity per se and its habituation are quite different depending upon the classes of stimuli used. These results show that, both by the criteria of initial responsiveness and habituation of that responding, animals receiving lesions of the caudate nuclei or the frontal cortices as adults are distinctly different, i.e., much more hyper-reactive, than either intact cats or those receiving comparable lesions as kittens. The rank ordering of groups, however, still remains, i.e. INT showed the most rapid habituation followed by BFr and, finally, by BAc. This seems to confirm that both frontal areas and the caudate nuclei are involved in the regulation of behavioral reactivity. (USPHS Grants HD-05958, MH-07097 and HD-94612).

144 OBSERVATIONS ON THE INTRINSIC ORGANIZATION OF THE RAT SUBSTANTIA NIGRA. C. J. Wilson*, S. J. Young* and P. M. Groves. Dept. Psych., Univ. Colo., Boulder, CO 80309.

Light and electron microscopic observations, and auto- and cross-correlational analyses of extracellularly recorded spontaneous neuronal activity have been used to investigate the intrinsic organization of the rat substantia nigra.

Most of the neurons of the substantia nigra, pars compacta are of medium size. Each possesses two sets of dendrites, one which descends into pars reticulata, and one which forms a disk-like dendritic field within the pars compacta, where it overlaps extensively with those of other pars compacta neurons. Auto-correlation histograms computed from spontaneous spike trains of pars compacta neurons are characterized by an 80-250 msec. period of low firing probability which follows generation of an action potential. Cross-correlations between simultaneously recorded pars compacta neurons indicate that nearby cells inhibit each other with a similar time course. A possible substrate for both of these phenomena is suggested by electron microscopic investigation of material taken from animals treated with 5-hydroxydopamine, which reveals the presence of monoaminergic dendro-dendritic synapses in the pars compacta.

In contrast, neurons of pars reticulata show a much shorter (15-45 msec.) post-firing inhibitory period. The majority of these show a tendency toward regular firing, and no tendency to inhibit each other. They probably correspond to the medium- and large-sized pars reticulata neurons seen in Golgi-stained preparations. A third less common neuron firing in a bursty pattern may correspond to the nigral interneuron. Cross-correlations between these and the more common pars reticulata neurons suggest that they may be arranged in a recurrent inhibitory network.

Supported by grant DA 01467 from the National Institute on Drug Abuse and Research Scientist Development Award K02 MH 70706 from the National Institute of Mental Health.

BRAIN METABOLISM AND NUTRITION

145 REHABILITATION FOLLOWING MALNUTRITION: MORPHOLOGICAL STUDIES OF THE RAT CEREBRAL CORTEX. Ana G. Angulo-Colmenares. Department of Anatomy, Boston University School of Medicine, Boston MA 02118

The effects of protein malnutrition and the possibility of rehabilitation have been studied in the somatosensory cortex of the rat. Animals were malnourished by giving their mothers an 8% casein diet beginning on day 10 of gestation and continuing until 20 days after birth. At this time rehabilitation was attempted by: a) feeding the animals a 24% casein diet, b) leaving the pups with their mother until they were 40 days old and c) reducing the litter size from 8 to 4 pups. Control animals were fed a 24% casein diet throughout the experiment. Observations were made on tissue from animals 20, 40 and 70 days old fixed by vascular perfusion of aldehydes. For 20 and 40 day animals the somatosensory cortex from one hemisphere was embedded in Araldite, and that from the contralateral side was processed for Golgi staining. Previous work (Colmenares, Neuroscience Abstracts, 2: 210, 76) on 20 day animals showed a reduction in the size of the cerebral hemispheres, in the thickness of somatosensory cortex, and in the proportion of tissue occupied by neuropil. In the latter case the difference was greatest in the upper half of layer II, less in the lower part of layer II and not statistically significant in layers III and IV. I now report on further analysis of 20 day old animals and observations on rehabilitated 40 and 70 day animals. At 40 days the length and width of the cerebral hemispheres, although nearer control values than at 20 days, are still significantly different. The thickness of the cerebral cortex is no longer significantly reduced and neither is the percent neuropil in layer II. The cell body volume of layer II neurons was measured in 1 μm plastic sections. In the 20 day old animals it is reduced by 23% ($P<.05$) and at 40 days a 12% difference in the rehabilitated animals is no longer statistically significant. In Golgi preparations the thickness of the apical and basal dendrites of layer II pyramidal neurons was measured at a distance of 20 μm from the center of the cell body. At 20 days of age the thickness of both apical and basal dendrites is reduced in the malnourished animals by 13% ($P<.025$) and 16% ($P<.005$) respectively. At 40 days, following rehabilitation, the thickness of both kinds of dendrites has improved to 12 and 13% respectively and the differences were still statistically significant ($P<.05$ and $P<.01$). At 70 days, figures are available only for cerebral hemisphere measurements. The length in the rehabilitated animals is 99% of controls, the width 98% and the height 100%. None of these differences is statistically significant. **These observations suggest the possibility that a normal structure of the cerebral cortex may be achieved following early malnutrition if adequate rehabilitation is provided following the stage of deprivation.**

146 **THE COMPARATIVE EFFECT OF HYPO- AND HYPER-OSMOTIC BLOOD PLASMA UPON BIOCHEMICAL CHANGES IN BRAIN TISSUES.** **Claude F. Baxter and Roger A. Baldwin*. Neurochemistry Laboratories, V.A. Hospital, Sepulveda, CA 91343, and Dept. of Psychiatry, UCLA School of Medicine, Los Angeles, CA 90024.**

Hypo- and hyper-osmotic changes in the blood plasma of the toad (Bufo boreas) in vivo are accompanied by corresponding depression and elevation of most "nonessential" amino acids, some "essential" amino acids, some amines, urea and ammonia in the brain tissues. In general, hypo-osmotic plasma conditions decreased and hyper-osmotic plasma conditions increased amino acids and their related compounds. Exceptions to this rule are methionine, isoleucine and leucine, which are elevated, and 3-methylhistidine, the level of which is depressed by osmotic changes in either direction; phenylalanine, lysine and to some extent histidine are elevated under hypo-osmotic and depressed by hyper-osmotic plasma conditions. The most striking change in brains of the hypo-osmotic toads is the depression of tyrosine levels to approximately one-third of their normal values. The patterns of changes in brain tissues indicate that different mechanisms regulating steady-state levels of amino acids and related compounds are affected by hyper- and hypo-osmotic conditions in the toad. These results have provided clues for biochemical mechanisms in brain, liver and other organ systems that may be susceptible to direct or indirect osmotic regulation. Some of these mechanisms have been or are being tested, and the results of these experiments will be presented.

147 EXTRACELLULAR POTASSIUM CHANGES DURING ISCHEMIA IN GERBILS. William F. Blank. NIH, Bethesda, MD 20014.

Extracellular potassium concentration (Ko) was measured continuously in gerbil cortex during ischemic insults caused by occluding one or both carotid arteries. Exponential increases in Ko were seen which resembled cortical spreading depression and which usually reached valves in excess of 100 mM. The fall in potassium after reversal of the insult occurred in two stages. A slow initial component occurred which may represent washout of Ko during re-establishment of blood flow. Following this the fall of Ko accelerated and could be described by a single exponential equation with a rate constant of about 0.03 per second. Undershoots of Ko of up to 0.5 mM below control levels (mean 3.0 mM) were frequently seen after reversal. With repeated or prolonged insults, the maximum rates of Ko rise decreased; the rate constants of the fall decreased; the Ko equilibrated at higher levels after reversal of the insult; and the undershoots disappeared.

Portions of the gerbil cortex were frozen with a dry-ice/acetone mixture in order to produce gliosis. Neurons were not present within the areas of astrocytic proliferation. Ko changes during ischemia were measured in the gliotic cortex and in normal cortex nearby. The changes of Ko in the normal portions of the cortex were similar to those described above. The Ko rise in the gliotic cortex was linear. The mean rate of rise was 3.7 mM/min. The kinetics of the fall following reversal was similar to that seen in intact cortex, however, undershoots were not seen and the rate constants were greater.

It is postulated that the presence of neurons is necessary for the occurrence of the exponential rise of Ko and the undershoot of Ko following reversal. The exponential rise probably occurs because of increased neuronal membrane permeability due to massive transmitter release. The linear rise in gliotic cortex is probably due to inhibition of Na^+-K^+ ATPase of the astrocytes (see Blank, W. F. and Kirshner, H. S., Brain Research 123: 113-124, 1977).

148 A GENETIC PREDISPOSITION TO THIAMINE DEFICIENCY IN FOUR PATIENTS WITH THIAMINE-DEFICIENCY DEMENTIA. John P. Blass and Gary E. Gibson. UCLA Medical School, Los Angeles, California 90024.

The Wernicke-Korsakoff syndrome is a well-characterized neuropsychiatric disorder associated in its chronic form with dementia, deficient short-term memory, and confabulatory psychosis. It is known to be due to a lack of dietary thiamine, but the rarity of this syndrome and its ethnic distribution suggest that genetic factors also play a role in its pathogenesis. We therefore studied the properties of transketolase (EC 2.2.1.1), a thiamine-dependent enzyme which is known to be affected early in thiamine deficiency, in cultured skin fibroblasts from four patients with typical chronic Wernicke-Korsakoff syndrome and six controls. Cultured fibroblasts are known to maintain the genome of the individual from whom they were derived during serial passages in culture which dilute out the material in the original biopsy by several million-fold. Thus abnormalities which persist in culture are presumably genetic rather than consequences of the disease or of malnutrition, alcohol abuse, etc. Transketolase activity was measured by a standard spectrophotometric coupled enzyme assay. Endogenous thiamine pyrophosphate (TPP) was removed from the enzyme by precipitation with $(NH_4)_2SO_4$ at pH 3.5.

Transketolase from all 4 patients showed an apparent K_m for TPP about one order of magnitude higher than for controls, although activity in the presence of saturating amounts of TPP was comparable to controls.

	K_m for TPP (μM)	Activity (Saturating TPP) (nmol/min per mg protein)
Controls	16 ± 2	17 ± 1
Patients	195 ± 31	22 ± 3
#1	281 ± 79	15 ± 3
#2	196 ± 45	22 ± 4
#3	156 ± 40	24 ± 1
#4	146 ± 45	27 ± 5

These results suggest that the patients have an inherited aberation in transketolase which would be a benign polymorphism on an adequate diet but predisposes them to developing thiamine deficiency on a diet marginal in thiamine. This "vitamin insufficiency syndrome" is an example of an inborn predisposition to the development of a neuropsychiatric disorder.

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149 EFFECT OF INSULIN INDUCED HYPOGLYCEMIA ON CEREBROSPINAL FLUID PRODUCTION. E. Fritschka*, M. E. Carey*, A. R. Vela*, J. J. Spitzer* (SPON: G. H. Ojemann). Dept. Physiol. and Neurosurg., L.S.U.M.C. Sch. Med., New Orleans, LA 70112.

The choroid plexus is thought to be the major source of CSF. Choroidal CSF is produced by an active secretory process involving $Na^{+}K^{+}$ ATPase. The choroid plexus also actively accumulates glucose and galactose from the CSF, perhaps by means of a low capacity sugar pump. (Progress in Brain Res 29:147,1967). No data are available concerning the effects of insulin induced hypoglycemia on CSF production (Vf).

Acute ventriculocisternal perfusion using mock cerebrospinal fluid containing blue dextran was used to measure CSF production in the anesthetized dog. Twelve mongrel dogs weighing approximately 10 kg were divided into two groups of 6 animals each. After an equilibrium period of 2 hrs to allow homogenous distribution of blue dextran in CSF basal control rates of cerebrospinal fluid production were obtained for 60 min. Following this control period the experimental animals received a constant intravenous insulin infusion of 1.5, 2.0 and 3.0 U/kg/hr. Blood samples and ventriculocisternal perfusate samples were collected on ice at 20 and 40 min for glucose and insulin determinations. Vf was estimated at a frequency of 20 min over the experimental time of 240 min. The control group was treated likewise, but received no insulin infusions.

Our experiments show that severe insulin induced hypoglycemia reduced CSF production. The Vf decreased significantly in all experimental animals and averaged after 240 min 40% of the control value. The Vf of the control group did not change significantly as determined by two way analysis of variance. The Vf in the experimental animals could be expressed as a logarithmic function of the glucose concentration of both the blood (r^2=.71) and the perfusate V (r^2=.83). Vf is approximately constant between blood glucose levels of 100 mg% and 20 mg%. Below 20 mg% a rapid decrease was observed. A linear regression of the mean Vf in each experimental period could be best fitted with a curve according to the formula Vf = .05 - .01x, x being the time in hours (r^2= .87). The mechanism of the Vf decrease remains to be elucidated.

150 EFFECTS OF VARIOUS PERIODS OF FOOD DEPRIVATION ON 5-HYDROXYTRYPTAMINE TURNOVER IN THE LATERAL HYPOTHALAMUS. K.M. Kantak,* M.J. Wayner and J.M. Stein*. Brain Res. Lab., Syracuse Univ., Syracuse, NY 13210.

The results from a recent study (Pharmac. Biochem. Behav., Kantak, et al, 1977) indicate a faster 5-hydroxytryptamine turnover rate in the lateral hypothalamus of 24 hr food deprived rats than in non-deprived rats. There was significantly more ^{3}H-5-hydroxyindoleacetic acid and ^{3}H-5-methoxytryptamine formed from ^{3}H-5-hydroxytryptamine in the 24 hr food deprived rats. In the present study, the periods of food deprivation were extended to include 48 and 72 hr food deprived rats. One hr following an infusion of ^{3}H-5-hydroxytryptamine, the lateral hypothalamus was perfused with physiological bacteriostatic saline for 40 min. Samples, which corresponded to 75-90 min post-infusion of ^{3}H-5-hydroxytryptamine, were analysed by thin layer chromatography for estimation of ^{3}H-labelled 5-hydroxytryptamine, 5-hydroxyindoleacetic acid, 5-methoxytryptamine, 5-methoxytryptophol and 5-methoxyindoleacetic acid. These results show that 5-hydroxytryptamine turnover is still enhanced as a result of food deprivation up to 72 hr. There was significantly more ^{3}H-5-hydroxyindoleacetic acid and ^{3}H-5-methoxyindoleacetic acid (the acid metabolite of 5-methoxytryptamine) in the 72 hr food deprived rats.

151 EFFECT OF AN 8% CASEIN DIET ON THE DEVELOPMENT OF NEURONS IN SUBCORTICAL FORMATION. Thomas L. Kemper* and Shelley Drazen* (SPON: W. Bruce Warr). Dept. Neuropathology, Boston City Hospital and Worcester Foundation for Experimental Biology, Boston, Mass. 02113.

Numerous studies have shown an effect of a low protein diet on the growth and maturation of the cerebral cortex. However little attention has been paid to the subcortical formations. In the present study the effects of dietary protein restriction was determined on rapid Golgi-impregnated neurons of the striatum, an example of a nucleated formation, and the nucleus of the diagonal band of Broca, an example of reticular formation.

Albino rats were conceived and suckled by mothers on an 8% Casein diet and controls on an isocaloric 25% Casein diet. After weaning the pups were maintained on their respective diet ad libitum. Six animals from each group were sacrificed at 90 days of age and the brains perfused in situ with 10% formalin, blocked and post fixed in rapid Golgi solution. Following silvering they were dehydrated, imbedded in LVN, and serially sectioned. Dendritic length and spine density measurements were made with an ocular reticle. In the striatum the spine-rich neurons of the 8% casein diet animals, as compared to controls, showed 9% decrease in spine density (1.96± 0.13 S.D.M. vs 2.16± 0.05 S.D.M. spines/micron) and a 2% decrease in dendritic length (141± 9 S.D.M. vs 144± 5 S.D.M. microns); the spine-free and spine-poor stellate cells showed a 4% decrease in dendritic length (110± 7 S.D.M. vs 115± 21 S.D.M. microns); and the large neurons a 3% decrease in dendritic length (253± 36 S.D.M. vs 262± 53 S.D.M. microns). The diagonal band neurons showed a 3% decrease in dendritic length (288± 15 S.D.M. vs 298± 15 S.D.M. microns). As compared to our previous study of dendritic and synaptic spine development in the visual cortical and cerebellar neurons (Brain Res. 103: 221, 1976) these phylogenically older and more conservative subcortical formations showed a correspondingly less striking effect of the 8% casein diet.

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152 INCREASED CEREBRAL BLOOD FLOW ELICITED BY STIMULATION OF FASTIGIAL NUCLEUS OF CEREBELLUM. Eric MacKenzie*, Masahiro Mori*, and Donald J. Reis (SPON. D. Park). Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021.

Electrical stimulation of the rostral medial pole of the fastigial nucleus (FN) of cat, monkey, and dog elicits a marked elevation of arterial pressure (AP). the fastigial pressor response (Miura and Reis, Am J Physiol 219:1330, 1970). We sought to establish if FN stimulation would also increase cerebral blood flow (CBF), as previously proposed (Doba and Reis, J Physiol 227:729, 1972). Focal CBF in the parietal cortices was measured by a hydrogen clearance technique in 42 normocapnic rabbits anesthetized with urethane, paralyzed and ventilated. Variations in AP produced by phenylephrine or controlled bleeding demonstrated an autoregulatory curve in rabbit comparable to that of other species. Electrical stimulation of FN at 5X current threshold for an elevation in AP substantially increased CBF. The increase was linearly dependent on the final AP. Thus, at a mean AP of 120 mmHg, for example, the CBF in unstimulated animals (AP elevated by phenylephrine) was 93±5 ml/100 g.min, during FN stimulation it increased to 148±15 ml/100 g.min (n=20, P<0.01). The increase in CBF was elicited only from pressor zones of FN and exhibited comparable stimulus frequency and response characteristics to the pressor response. Elevation of AP produced by electrical stimulation of pressor areas in the medullary tegmentum did not increase CBF. Abolition of the increase in AP by spinal cord transection did not inhibit but rather enhanced the response. Cervical sympathectomy did not affect it. We conclude that the cerebellum contains a neural system, not previously recognized, which can elicit profound vasodilatation of the cerebral circulation, probably through an intrinsic neuronal system of the brainstem.

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153 HISTIDINE-INDUCED ELEVATION OF BRAIN CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE ASSOCIATED WITH ELEVATED BRAIN HISTAMINE IN RATS. T.M. McMurray*, E. Orr*, A. Qureshi*, and B. Eichelman. (SPON: R.F. Keesey). Laboratory of Behavioral Neurochemistry, Dept. Psychiat., Univ. Wisconsin and VA Hospital, Madison, WI 53705.

Recently, we have reported that feeding rats an L-histidine supplemented diet causes a decrease in lipogenic enzymes (Eichelman et al., 1977) and an induction of cholesterol biosynthesis(Qureshi et al., 1977) in both brain and liver. Previous work on liver lipogenic enzyme systems suggested that these effects might be mediated by increases in cyclic adenosine 3',5'-monophosphate(cAMP) levels. The present studies describe the effect of different nutritional states(employing histidine loading) on the levels of brain cAMP.

Rats were maintained acutely for three days and chronically for twenty-one days on a diet enriched 5% with L-histidine. To maximize enzyme levels under study, the rats were fasted for two days and refed for three days prior to sacrifice. In both the acute and chronic studies there was an increase of brain cAMP levels to 60-100% over control levels in groups fed a histidine enriched diet. Rats injected with L-histidine (500 mg/kg, ip) showed 100% increases in brain cAMP levels after two to three hours. Decreases in brain lipogenic enzymes were proportional to the increases in brain cAMP levels over the three hour period.

Findings that histamine plays a role as a putative neurotransmitter and has also been shown to increase intracellular cAMP through H2 receptors in vitro(Busse & Sosman, 1976) suggested a mechanism whereby brain cAMP might be increased following histidine loading. Our results showed that histamine levels under the above conditions were increased similar to the cAMP increases.

Brain levels of cAMP following three day feeding and three hour injections of urocanic acid(an irreversible histidine metabolite) showed a slight increase.

These results are compatible with the hypothesis that histidine induced changes in brain lipogenic enzymes and cholesterol biosynthesis are mediated through histamine induced increases in brain cAMP. (This work was supported by grants from the Medical Research Service of the Veterans Administration Hospital and intramural grants from the University of Wisconsin.)

154 PRESENCE OF DIHYDROFOLATE REDUCTASE IN RAT BRAIN DURING EARLY DEVELOPMENT. J.C. Mendible* and L.A. Ordóñez. Lab. Neuroquimica y Comportamiento. I.M.E. Apdo: 50587. Sabana Grande. Caracas-Venezuela.

Folates are involved in metabolic processes which seem to be essential during early mammalian brain development, as shown by increased total folate levels (L.D. McClain and W.F. Bridgers, J. Neurochem 17:763, 1970), increased serine-tetrahydrofolate 5,10-hydroxymethyl transferase activity (W.F. Bridgers, J. Neurochem 15:1325,1968) and increased methylene reductase activity (L.A.Ordóñez and O. Villarroel, J. Neurochem 27:305,1976) in developing brain when compared to adult tissue. These changes could reflect the involvement of folates in the generation of thymidine requiered for deoxyribonucleic acid synthesis. In thymidine synthesis, tetrahydrofolic acid (THFA) is oxydized to dihydrofolic acid and it must be reduced back to THFA by Dihydrofolate Reductase (DR) before being able to participate again in metabolism. This enzyme has been reported to be absent from adult brain (D.R. Makulu, et al, J. Neurochem 21: 241,1973); we have studied the activity of this enzyme in rat brain and liver from five days before birth (-5 days) to adult. DR is present in fetal brain (-5 days; S.A: 0.414 ± 0.034 umol/h.mg) and decreases continuosly until becoming non-detectable in the adult. The enzyme is still detectable at 20 days of age (S.A. 0.045 ± 0.009 umol/h.mg). In contrast, the liver enzyme shows a constant activity from -5 days until adult (~0.27 umol/h.mg). At 0 days of age cerebellum DR activity is lower (0.084 ± 0.007 umol/h.mg) than that of the rest of the brain (0.120 ± 0.011 umol/h.mg) but in the former area this activity remains relatively constant until day 20 while in the rest of the brain it decreases rapidly with time. In both brain areas no activity is detectable in the adult.

These result support the importance of folates during early brain development probably through their involvement in thymidine synthesis and could explain the neurological disorders reported in humans and other animals associated with folate deficiencies during early development.

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155 BRAIN AND PERIPHERAL UTILIZATION OF LABELLED TRYPTOPHAN IN PROTEIN MALNOURISHED RATS. Maravene Miller*, Oscar Resnick*, J. Patrick Leahy* and Peter J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Ma. 01545.

The utilization of IP injected ^{14}C-tryptophan (^{14}C-trp) for incorporation into protein in brain and peripheral tissues from birth to 21 days was examined in rats fed a normal (25% casein) or a low protein (8% casein) diet. The malnourished group, whose dams received the low protein diet 5 weeks prior to conception, showed significant alterations in the incorporation and percent incorporation of ^{14}C-trp into protein on the day of birth as compared to the normal group (table below). The lower incorporation of ^{14}C-trp into protein by the malnourished rats was also noted at the subsequent ages examined (day 5 to day 21).

This altered pattern of incorporation of ^{14}C-trp by the malnourished rats may be due, in part, to their higher concentrations of free plasma trp at these ages as reported by Miller et al. (Soc. Neurosci. p. 586, 1976). The injected trp may have less affinity for tissue incorporation due to the higher concentration of endogenous free plasma trp seen in these animals (Miller et al. Exp. Neurol., in press).

^{14}C-Trp Uptake on Day of Birth (Mean ± S.E.)[1]

Diets:	8%	25%
(n)	5	5
	Tissue Homogenates (dpm x 10^2/g)	
Telencephalon	1185 ± 56	1427 ± 170
Brainstem	1293 ± 96	1490 ± 139
Liver	2450 ± 291	2676 ± 287
Kidney	3024 ± 413	3181 ± 395
	Protein Precipitates (dpm x 10^2/g)	
Telencephalon	52 ± 4[a]	153 ± 19
Brainstem	61 ± 5[a]	175 ± 17
Liver	291 ± 15[a]	968 ± 135
Kidney	239 ± 41[b]	705 ± 173
	Percent Incorporation into Protein	
Telencephalon	4 ± 0[a]	11 ± 1
Brainstem	5 ± 0[a]	12 ± 1
Liver	12 ± 2[a]	36 ± 1
Kidney	8 ± 1[a]	21 ± 2

[1] 20 minutes post-injection

[a] $p < 0.001$

[b] $p < 0.02$, 2-tailed t-tests

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156 DEPLETION OF CYTOCHROME OXIDASE IN BRAIN MITOCHONDRIA FROM THE MOTTLED MOUSE MUTANT. Donald L. Rezek* and Cyril L. Moore* (SPON: G. A. Barr). Dept. Neurology, Albert Einstein Coll. of Med., Bronx, NY 10461.

A genetic defect prevents the male mottled mouse (Mo^{br}/+) from the normal utilization of copper. Among the enzymes most directly affected is brain mitochondrial cytochrome oxidase. Since this enzyme is essential for respiration and oxidative phosphorylation, it is noteworthy that the amount of the spectrophotometrically measureable enzyme decreases from birth to when the animal dies at about 14 days of age. In brain mitochondria of 2-4 day old animals, the ratio of the amount of cytochrome oxidase in the affected animals to that in the normal controls is 0.48; by 6-8 days of age, it is 0.43; and by 11-14 days of age, it is only 0.14. The liver, which sequesters much of the body's copper, is not nearly as affected as the brain; the respective ratios in the liver are 0.88, 1.03, and 0.73. The rate at which added cytochrome c is oxidized in brain homogenates is about 30% of normal. The study emphasizes the importance of copper as an intregal part of cytochrome a_1-a_3 in the brain and suggests that depletion of cytochrome oxidase in the mouse and in victims of the human genetic disease, Menkes kinky hair syndrome, may be related to the retardation and death caused by these diseases. (Supported by NIMH training grant MH 06418.)

157 EFFECTS OF SODIUM OCTANOATE ON BLOOD-BRAIN BARRIER PERMEABILITY.
Doris A. Trauner and Jack de la Torre. Univ. of California, San Diego, CA 92103 and Univ. of Chicago, Chicago, IL 60637.

The short chain fatty acid (SCFA) sodium octanoate causes hyperventilation, coma, seizures and electroencephalographic slowing in experimental animals. The mechanism by which this substance exerts its toxic effects is unknown. The rapidity with which octanoate produces such alterations suggests that this substance may exert some effect on blood-brain barrier (BBB) permeability. The present report describes preliminary results concerning BBB alterations in rats given intraperitoneal (IP) injections of sodium octanoate, 2 ml of a 1 M solution.

A modified glyoxylic acid histofluorescence technique (SPG method) was used to study vascular integrity in rats pretreated with octanoate and followed in 2-4 hours with injection of the monoamine oxidase inhibitor, nialamide, and L-dopa. Following L-dopa injection, there was marked diffusion of fluorescence around brain capillaries, particularly in subcortical white matter. Diffusion was also seen around brainstem neurons and was striking around cell bodies in the locus coeruleus and magnacellularis. Fluorescent diffusion was prominent around the choroid plexus as well. Diffusion of catecholaminergic terminals was also observed throughout the brain and markedly around the nucleus rotundo and stellatocellularis and the periventricular nucleus of the hypothalamus. It is of interest to note that this catecholaminergic diffusion appeared more severe in brain regions in close proximity to the ventricular system.

Fluorescence was confined within capillaries and no diffusion was seen in animals injected with nialamide and L-dopa but not pretreated with octanoate.

These data suggest that octanoic acid alters the permeability of the BBB and may produce marked cerebral metabolic changes as a result of this permeability change.

CEREBELLUM

158 FASTIGIAL UNIT ACTIVITY DURING WRIST MOVEMENTS IN PRIMATES. Antonio Bava* and Robert Grimm. Neurological Sciences Institute, Portland, Oregon 97209.

Crab-eating macaques (Macaca irus) were trained to make a series of quick (250-500°/sec), self-paced 45° flexion or extension movements of a manipulandum (stiffness: 0.86-2.9 kg. cm/rad) in order to examine the proposition that fastigial n. of cerebellum provides a fast feedback pathway to thalamus for spinal information regarding movement. Fastigial n. receives a rich supply of excitatory mossy fiber (MF) and climbing fiber (CF) collaterals carrying spinal and suprasegmental activity destined for anterior lobe. As fastigial n. also projects to ventrolateral (VL) n. of thalamus, Ia, Ib, and group II activity could reach motor cortex by this route. Accordingly, our hypothesis was that fastigial neurons participating in a voluntary movement would alter their discharge after a movement begins.

From a sample of 139 units in 20 tracks, we divided fastigial neurons into four groups: slow-firing units (1-10 Hz; 80% of the sample); tonic units (20-50 Hz; 14%); "silent" units (only recruited during a movement; 5%); and high frequency, short duration burst units (1%; presumably interneurons). Slow-firing units characteristically increase their discharge rate 200-300 msec prior to the onset of the first of a series of repeated movements (performance rate 1/sec). However, in each subsequent performance in a series, such units only discharge after force begins to rise but before handle displacement. After the last movement of a series is completed, 50% of slow-firing units discharge in concert with proximal-axial activity returning the limb to a rest position. Tonic units either increase (20%) or decrease (80%) their activity after movement begins. "Silent" units, active only with movement, always discharge before a first performance movement, thereafter decrementing as the monkey repeats the task.

These data establish that fastigial neurons are recruited before or after a movement begins depending on the context. For single movements or the first-in-a-series, "silent" and slow-firing units discharge before movement; thereafter, discharges follow (40-200 msec) the onset of similar movements serially repeated (1/sec). This rule does not apply to tonic units: they only alter their activity after a movement begins regardless of occurrence order. Where unit activity could be correlated with performance metrics, it appears that a force-velocity algorithm or changes therein and not a position measure is the work of this nucleus. The different attention given by the nucleus to single vs. repeated movements, and, tonic unit inhibition during movement are novel findings.

159 THE MORPHOLOGY OF THE FASTIGIAL NUCLEUS IN THE RAT. Alvin J. Beitz* and Victoria Chan-Palay (SPON: Nell B. Cant). Dept. Anat., Harvard Medical School, Boston, MA 02115.

The present investigation is aimed at describing the organization of the fastigial nucleus in the rat utilizing several variants of the Golgi technique and Nissl and Weigert preparations. A three-dimensional model of the fastigial nucleus was made from reconstructions of serial coronal sections stained with the Nissl method. This illustrates both the shape of the nucleus and the location and extent of the three nuclear subdivisions as described by Korneliussen (1968). The middle subdivision forms the largest portion of the nucleus and extends most rostrally while the dorsolateral protuberance and the caudomedial portion comprise smaller more caudal subdivisions of the nucleus.

In order to determine the frequency distribution of neuronal sizes within the fastigius, measurements were made of the lengths, widths and areas of 550 neurons drawn from Nissl-stained sections of the three divisions in a single animal. Two classes of neurons are present: small neurons with cross sectional areas less than 180μm^2 and larger cells with areas greater than 180μm^2. The larger neurons range in width from 10 to 22μm and in length from 18 to 35μm and are most numerous in the dorsolateral protuberance. The middle subdivision contains a mixture of large and small cells. Small neurons predominate in the caudomedial subdivision of the nucleus and range in width from 5 to 16μm and in length from 10 to 24μm. In Golgi preparations, both the small and large neurons have two major types of dendritic patterns: either the cells are truly multipolar or they have dendrites oriented in two directions. Histograms were prepared relating the numbers of neuronal perikarya in both frontal and horizontal sections with their ellipticity ratios (ER width:length). These histograms indicate that there are two basic shapes of neuronal perikarya in the rat fastigial nucleus: almost round (ER<1:1.5) and elliptical (ER>1:1.5). When the locations of round versus elongated cells were charted, three distinct rostrocaudal zones became evident within the nucleus: a medial and lateral zone with predominantly elliptical neurons and an intermediate round cell zone. All impregnated neurons in a set of horizontal Golgi-Kopsch serial sections were drawn to study cellular organization in detail. Elongated neurons in the medial and lateral zones of the nucleus are fusiform with their cell bodies and dendrites oriented predominantly in a rostrocaudal direction. The rounded cells of the intermediate zone are multipolar with their perikarya and dendrites arranged in a more mediolateral direction. Elongated neurons in the caudomedial subdivision displayed a distinct rostromedial-to-caudolateral orientation. (Supported by Neuroanatomy Training Grant NS 05591 to Dr. S. L. Palay and NIH Postdoctoral Fellowship NS 05688.)

160 OLIVARY UNIT ACTIVITY AND EFFECT OF MICROSTIMULATION DURING LOCOMOTION. C. C. Boylls, Jr., Biomed. Res. Lab., E. E. Dept., U. Maryland, College Park, MD 20742.

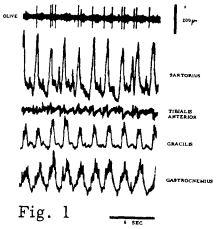

Fig. 1

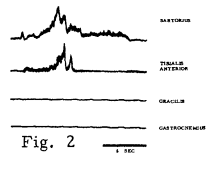

Fig. 2

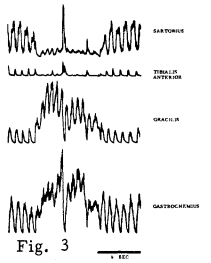

Fig. 3

Thalamic cats were prepared for slow treadmill walking (0.3-0.7 kph), with EMG's ("integrated" in records below) led from muscles of one hindlimb. Using antidromic field techniques, we then inserted tungsten electrodes into a portion of the contralateral MAO contributing climbing fibers to a parasagittal anterior lobe strip roughly 1.8 mm wide, adjacent to the midline. Here we found MAO unit activity to have but slight correlation with locomotor movements (fig. 1). However, MAO microstimulation (0.2 msec pulses, 5-30 hz), at levels just suprathreshold for cortical surface potentials (25-45 μa), was found to augment powerfully the contractions of limb flexors or extensors, with concomitant antagonist suppression (hindlimb fixed in fig. 2, walking in fig. 3). After stimulus cessation, such agonistic rebiasing required many seconds to decay.

MAO activity thus appears to affect the "carriage" of locomotion over many step cycles, hence the lack of unit activity locked to single cycles. Olivary postural biasing appears encoded in terms of multijoint, agonistic muscle groups, possibly synonymous with the agonists of spinal reflexes. Perhaps each such group is represented by a climbing fiber strip.

I thank Drs. F. E. Zajac and J. D. Cowan for the opportunity to conduct this study, Dr. N. H. Barmack for advice and encouragement, and Mssrs. D. Dungan and C. A. Twigg for expert technical assistance. Supported by NIH grant NS 11518 and postdoctoral fellowship 1 F32 NS 05568.

161 THE VENTRAL PARAFLOCCULUS: A SITE OF VISUAL CORTICAL AND TECTAL INPUT. Richard A. Burne and Donald J. Woodward. Dept. Cell Bio., Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235.

This study was undertaken with the general aim of investigating areas in rat cerebellum which receive visual information.

In this report we describe anatomical and electrophysiological evidence that the ventral parafloccculus (vpf) receives information from the visual cortex and superior colliculus via relays in the pons and inferior olive. The techniques of retrograde transport of horseradish peroxidase (HRP) and orthograde transport of tritiated L-leucine were employed to determine pathways from cortex and tectum to the cerebellum.

Injections of ^{3}H-leucine into areas 17, 18, and 18A of visual cortex resulted in overlapping terminal labeling within the rostral lateral pontine grey. In addition, two injections confined within area 17 gave labeling in a medial rostral pontine group which suggests a more detailed topography. Injections of leucine into superior colliculus gave ipsilateral labeling in lateral areas of the middle pons, and the ipsilateral medial accessory olive including the beta nucleus.

Injections of HRP in vpf yielded labeled neurons which revealed a bilateral projection (contralateral predominance) from medial and lateral neurons in the rostral pons, and a contralateral projection from medial accessory and principle olive. Experiments with simultaneous injections of HRP and leucine revealed in adjacent sections considerable overlap of leucine labeled terminals from visual cortex and superior colliculus onto HRP labeled pontine and inferior olive neurons which project to vpf.

Unit recordings and post-stimulus-time-histograms from each of 50 Purkinje cells in vpf showed evidence of mixed excitatory-inhibitory mossy fiber input (36 cells) or pure inhibitory responses (14 cells) following cortical or tectal stimulation with concentric bipolar electrodes (0.1 ms, 0.2-0.4 mA, 2-10 Hz). Mean latency to onset was 10.2 ± .4 msec for cortex and 7.8 ± .3 msec for tectum.

We conclude that the ventral parafloccculus is a cerebellar area which receives a convergence of strong visual input from visual cortex and information from superior colliculus.

(This study was supported by grants NSF GB43301 and NIH NS13225 to D.J.W.).

162 EFFECTS OF COOLING OF THE CEREBELLAR NUCLEI ON THE MOTOR OUTPUT DURING FAST ELBOW FLEXIONS IN CATS. John E. Burton and John Kerch Engelhardt. Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010.

Cats were trained to perform ballistically initiated elbow flexions and prepared for local cooling of individual cerebellar nuclei. The structure of the motor output following the onset of movement is characterized by a silent period in the agonist (biceps) muscle and a burst of EMG activity in the antagonist (triceps), both of which coincide with the peak of the velocity. Local cooling of the ipsilateral dentate nucleus did not alter this structure. Cooling of the interpositus nucleus, however, delayed both the decline of the biceps activity and the onset of the triceps activity relative to the onset of the motion. Also, the biceps muscle was no longer silenced and the amount of triceps activity was reduced. As a result of these changes, the acceleration was prolonged and the peak velocity and displacement attained higher values. Cooling of the fastigial nuclei (bilateral) produced either a slight advance of the termination of the biceps activity or a delay with absence of silence depending upon the position of the thermode and/or the depth of the cooling. The triceps activity was usually delayed and the amount increased. The displacement was characterized by large oscillations. It is concluded that the output of both the fastigial and the interpositus nuclei contributes to regulate the amount and timing of the motor output during fast movements in cats. The results will be discussed in terms of the behavior of neurons of the cerebellar nuclei, red nucleus, and lateral vestibular nucleus during such movements. (Supported by NIH research grant number NS 11798 from NINCDS and by the Jerry Rosen Research Fund)

163 THE SOURCE OF CLIMBING FIBERS TO THE VESTIBULO-CEREBELLUM OF THE TADPOLE. S.L. Cochran and J.T. Hackett. Dept. Physiology, Univ. of Virginia Medical School, Charlottesville, Virginia, 22901.

Current research suggests that the inferior olive is the exclusive source of climbing fibers(CFs). However, an extra-olivary origin of CFs has been reported in the frog to project from the periphery to the auricular lobe("vestibulo-cerebellum") via the VIIIth cranial nerve. Our own experiments upon the frog (Cochran and Hackett, 1977) have failed to locate any extra-olivary source of CFs. This failure could be due to difficulties in sampling the minute auricular cerebellum of the frog. According to Larsell (1923,1967), the tadpole cerebellum is predominantly auricular. Therefore, we have investigated the source of CFs to this cerebellum. Electrophysiological experiments were performed upon isolated brains of premetamorphic Rana pipiens and Rana catesbeiana tadpoles. Purkinje cells(PCs) were recognized electrophysiologically by their characteristic antidromic, CF, and mossy fiber(MF) evoked responses. This recognition was anatomically verified by subsequently processing the tissue histologically following ejection of horseradish peroxidase(HRP) from the recording microelectrode. Direct CF-evoked responses were identified by their fixed latency, by their all-or-nothing character, and by their interposition between the directly-evoked antidromic response and the disynaptically-evoked MF response in the PC. Furthermore, intracellular recordings in evoked PCs uncover a single, large, fixed latency, all-or-nothing EPSP, the polarity of which could be reversed by depolarizing current injection. By repositioning the stimulating electrode, individual CFs could be traced along their trajectory through the brain. A linear correlation($r>0.9$; $p<0.001$)existed between the latency to PC activation and the distance of the stimulating electrode from the cerebellum, thereby demonstrating direct CF activation. CF activation could be found only along the lateral edge of the brain, past cranial nerves VII, VIII, and IX. At the level of the Xth cranial nerve, a CF's path could be traced caudally, ventro-medially and across the midline where large latency shifts in activation suggested other than direct CF stimulation. Some axons appeared not to cross the midline. Electron microscopic observations of the cerebellum reveal a cellular association characteristic of that attributed to the CF and PC in the tadpole(Cajal, 1911). We therefore conclude that the tadpole indeed possesses a CF afferent system and that even in a limbless, finless vertebrate, the caudal medulla has a unique relationship with the cerebellum in that only it innervates the PCs with CFs. The demonstration of this unique source of CFs in all vertebrates suggests that the functional contribution of the CF to cerebellar action is, to some extent, fixed in phylogeny. (Supported by NSF Grant BNS 74-01423 A02)

164 PONTINE PROJECTION TO CEREBELLAR CORTEX AND NUCLEI. J. Courville and G. Coulombe. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, H3C 3T8

The distribution of the pontine projection onto the cerebellar cortex and nuclei was studied with methods of anterograde degeneration and tritiated amino acid transport. Multiple large injections of L-leucine in cats demonstrate the course of the fibers and a terminal distribution appearing as roughly circular grain accumulations and restricted to the granular layer. The projection is abundant in the contralateral, lateral portion of the anterior lobe, in lobules VI, VII, simplex, paramedian and in Crus I, Crus II and paraflocculus. For vermian lobules VI and VII, an abundant bilateral distribution originates from each side of the pontine gray. The central region of the anterior lobe comprising the vermis and part of the intermediate zone is practically devoid of pontine afferents. After small localized injections in the pons, the grain accumulations are much less abundant. However, they always appear widely distributed to different cerebellar regions. Anterograde degeneration experiments confirm these data except for the quantitative differences for each side. This is interpreted as a result of a destruction of crossed fibers which occurs with unilateral lesions. Concerning a projection to the cerebellar nuclei, the evidence obtained is that grain counts are significantly augmented in the dentate nuclei, the difference being greater in the nucleus contralateral to the injection. Degeneration studies confirm this distribution and support the conclusion of a rather sparse distribution. In addition, it appears that this projection originates mostly if not exclusively, from the nucleus reticularis tegmenti pontis. Injections of horseradish peroxidase in the nuclei have yielded inconsistant results. Medially situated injections were not followed by any retrograde labeling of pontine cells. Laterally localized injections either showed few labeled cells or widespread distributions of a large number of labeled elements. These vagaries are attributed to variations in the uptake by fibers of passage and to differences in the degree of fixation among cases.

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165 TOPOGRAPHIC ORGANIZATION OF PROJECTIONS FROM THE DENTATE AND INTERPOSITUS NUCLEI IN THE RHESUS MONKEY: AN AUTORADIOGRAPHIC STUDY. K. Kalil. Dept. of Anat., University of Wisconsin, Madison, WI., 53706.

Injections of (^{3}H) proline were made into the dentate and interpositus nuclei of rhesus monkeys. Several different approaches were used to inject either an entire cerebellar nucleus or selected regions of the dentate and interpositus nuclei. All efferent pathways from the nuclei to the brainstem and thalamus were traced but particular attention was focused on the topographic organization of cerebellar projections to the inferior olivary complex and the ventral tier nuclei of the thalamus. The dentate nucleus projects upon the principal inferior olivary nucleus in a precisely ordered topography such that the ventral dentate terminates upon the ventral lamella of the olive and the dorsal dentate projects upon its dorsal lip. Moreover, these connections follow an exact and non-overlapping mediolateral topography. By contrast, when regions of the interpositus were injected with minimal encroachment into the dentate only the dorsal and medial accessory olivary nuclei were labeled and not the principal nucleus. Preliminary results suggest that this projection is also topographically organized. For example, when the entire interpositus was injected the entire medial accessory olive was covered with silver grains, but injections of only the dorsal or ventral regions of the interpositus resulted in selective labeling of the dorsal and medial accessory olive. Superimposed upon this point to point organization is an anterior posterior topography with caudal regions of the cerebellar nuclei projecting upon the rostral olive and vice-versa. As has been previously reported, these connections are overwhelmingly crossed with only a small corresponding ipsilateral projection.

The cerebello-thalamic fibers are also topographically ordered with the caudal and rostral regions of the cerebellar nuclei projecting respectively to the medial and lateral zones of the thalamus. The thalamic nuclei receiving the densest cerebellar efferents are the VLc, the VPLo and the VLo. In several cases nucleus X was labeled but even with massive injections of dentate and interpositus there were surprisingly sparse projections to the VA. There was also intense labeling of CL and the reticular nucleus. While the cerebello-thalamic pathways are mainly crossed there is nevertheless a small corresponding ipsilateral component. In some cases the terminal labeling in the VLc, VPLo and VLo appeared in striking narrow horizontal bands or strips across the ventral thalamic nuclei and in other cases as a discontinuous series of patches.

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166 PURKINJE CELL ACTIVITY IN EYE MOVEMENT AND FIXATION IN THE MONKEY VERMIS. Manabu Kase* and Hiroharu Noda Brain Research Institute, Depts. Physiol. Anat., Sch. Med., UCLA, Los Angeles, CA 90024

Single unit recordings were made from Purkinje cells (P-cells) in the vermis (Lobus VI and VII) of the monkey during saccadic eye movements and steady fixation. Most P-cells exhibited a considerably high level of simple spike discharges (55 - 100 Hz) which were interspersed by characteristic complex spike discharges (0.5 - 1.5 Hz). There were two classes of P-cells.

One class of P-cells showed changes in activity only with eye movements. These cells increased discharges, typically starting 10 - 20 msec before the initiation of a saccade and showing peak activity at about the middle of the course of the saccade. In a small number of P-cells, the firing decreased during a saccade with a time course similar to the transient increase. However, complete cessation of firing extending the entire duration of eye movement was seldom observed. Many P-cells in this class also showed preferred direction and the increase in activity was observed only when the saccade was in the preferred direction of the concerned cell. Although there was a clear relation between the duration of bursts and the size of associated eye movements, no relation was found between instantaneous firing rate within the burst and amplitude of accompanying eye movement.

The other class of P-cells also showed changes in activity during saccades. Discharges in these cells were completely suppressed during a saccade. Furthermore, the level of tonic discharges of the cells varied considerably (for example, 0 - 120 Hz in a cell) from one point of fixation to another, showing a higher level of activity when the gaze shifted to the preferred direction of the cell. The discharge rate was clearly a linear function of eye position in some cells, indicating that these cells convey information relevant to eye position in the orbit. (Supported by NIH Grant EY01051).

167 DISTRIBUTION OF VESTIBULAR NERVE FIBERS TO THE CEREBELLUM IN THE CAT. Gary E. Korte* and Enrico Mugnaini. Dept. of Biobehavioral Sciences, Univ. of Conn., Storrs, Conn. 06268

The cerebellar distribution of the vestibular nerve in the cat has been re-examined with silver degeneration techniques and in 1-2 um thick sections of plastic embedded material. The vestibular ganglion was manually destroyed via the bulla by twisting a dental burr into the internal meatus. After survival times of 3-6 days, the animals were processed for examination by the Fink-Heimer and Nauta-Laidlaw techniques or prepared for electron microscopy. The extent of the lesion was analyzed under the dissecting microscope and by light microscopic examination of 1-2 um thick sections of the plastic embedded remaining nerve stump.

The Fink-Heimer and Nauta-Laidlaw techniques revealed a conspicuous fiber bundle entering the cerebellar white matter and terminating in the granular layer of all folia of the nodulus and the ventral folia of the uvula on the ipsilateral side. Relatively fewer degenerating fibers were traced to the cortex of the ipsilateral flocculus. No projection to the paraflocculus, lingula, lateral cerebellar nucleus or any contralateral cerebellar region could be ascertained (some degenerating fibers were present in the fastigial nucleus, but we are still uncertain as to whether they pass by or give rise to a terminal arborization).

These observations were confirmed by light microscopic observation of 1-2 um thick plastic sections from specimens processed for electron microscopy. Electron microscopic controls are in progress.

In conclusion, a re-examination of the cerebellar terminal field of the primary vestibular fibers in the cat has shown that it is mainly represented by the cortex of the ipsilateral nodulus and uvula, and to a lesser extent by the cortex of the ipsilateral flocculus.

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168 $[K^+]_o$ RISE PRECEDES $[Ca^{++}]_o$ FALL DURING SPREADING DEPRESSION. R. P. Kraig, C. Nicholson and J. M. Phillips*. Dept. Physiol. & Biophys., N.Y.Univ.Med.Ctr., 550 First Ave., New York, NY 10016.

Recent experiments from this laboratory have used spreading depression (SD) to explore the capacity of the brain to reversibly undergo fluctuations in the extracellular ionic milieu. During SD in the cerebellar molecular layer of the catfish, Corydoras aneus, the $[Na^+]_o$ typically falls from 149 mM to 57 mM, and the $[Cl^-]_o$ falls from 137 mM to 47 mM while the $[K^+]_o$ rises from 2 mM to 35 mM (1,2). It has recently been established that Ca^{++} and K^+ fluxes are related in certain membranes (3,4) and also that correlated $[K^+]_o$ rises and $[Ca^{++}]_o$ falls occur in the brain during aminopyridine application (5), convulsive activity (6), repetitive stimulation, spreading depression and anoxia (7). We have utilized the slowly developing SD of the catfish cerebellum to investigate the temporal relationship between $[K^+]_o$ and $[Ca^{++}]_o$ changes. Pairs of ion-selective micropipettes were fabricated from theta-tube capillaries and sensitized with Ca^{++}-selective liquid ion exchanger (8) or Corning 477317 K^+ exchanger. SD was initiated in the exposed catfish cerebellum by a microinjection of 1M KCl.

Baseline $[K^+]_o$ was about 2 mM and baseline $[Ca^{++}]_o$ about 1.6 mM. Thus the two ISM were approximately equal in sensitivity to ion changes. During SD the $[K^+]_o$ always began to rise several seconds before the $[Ca^{++}]_o$ began to decrease. At the peak of SD the $[Ca^{++}]_o$ decreased to about 25% of its baseline value. The resolution of Ca^{++} and K^+ ISMs was such that $\pm$ 0.1 mM could be detected. In no instance did $[Ca^{++}]_o$ begin to fall before the $[K^+]_o$ began to rise.

We conclude that a prolonged neuronal Ca^{++} influx, as manifested by a decrease in the $[Ca^{++}]_o$, does not precede the well established rise in $[K^+]_o$ during SD. Rather the marked delay in the $[Ca^{++}]_o$ decrease suggests at least two partially separable events occurring during SD: a rise in the $[K^+]_o$ and a subsequent fall in the $[Ca^{++}]_o$.

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169 STRUCTURE OF THE PURKINJE CELL DENDRITIC MEMBRANE DURING SYNAPTOGENESIS. D. M. D. Landis and T. S. Reese. Mass. General Hosp., Boston, MA 02114; LNNS, NINCDS, NIH, Bethesda, MD 20014.

Freeze-fractured Purkinje dendritic spines in mature mice have aggregates of intramembrane particles arrayed on their external membrane leaflet at synaptic junctions with parallel fibers. We investigated the formation of this postsynaptic specialization and attempted to relate it to parallel fiber synaptogenesis by freeze-fracturing cerebellar cortex during postnatal development. Criteria for recognizing Purkinje dendrites, especially in younger mice, were established by tracing their continuity with cell bodies. Dendritic segments distal to the region of apparently mature spines were examined in detail because this is where spine formation and synaptogenesis occur. However, the membrane structure of distal segments was generally like that of the rest of the Purkinje dendrites and cell bodies, except that coated vesicle openings were more frequent; these proved to be a reliable marker to identify comparable regions of Purkinje dendrites in thin sections and replicas. Infrequently, aggregates of particles which resembled but were smaller than postsynaptic specializations on mature Purkinje spines were found on the external leaflets of dendritic shafts, usually distal to the zone of well-formed spines. The membrane at these aggregates was sometimes indented by varicose processes, but the course of these unidentified processes was usually different from the adjacent parallel fibers. Thin sections of distal dendritic segments revealed numerous junctions between parallel fibers and dendritic shafts which, like mature synapses, were characterized by bands of submembrane fuzz in the Purkinje dendrites. Parallel fibers often contained a few synaptic vesicles near these junctions. Since parallel fibers do not contact Purkinje dendritic shafts in adult animals, the junctions with the dendritic shafts observed during development either must become or be replaced by spine synapses. The clear disparity between the large number of these junctions in thin sections and the small number of particle aggregates seen on distal dendrites with the freeze-fracture technique suggests that the vast majority of these junctions do not have the membrane structure of a mature spine synapse. Thus, the postsynaptic particle aggregates which characterize Purkinje spines are probably acquired after the formation of spines begins. If the junctions on Purkinje shafts are developing synapses, then early forms of these synapses lack intramembrane particles; an alternative interpretation of our data is that synapse formation begins later than spine formation. In this case, the junctions on the Purkinje shafts could not be regarded as precursors of synaptic junctions.

170 CALCIUM DENDRITIC SPIKES IN THE MAMMALIAN PURKINJE CELLS. R. Llinás, M. Sugimori* and K. Walton. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Electrical excitability of Purkinje cell dendrites in the molecular layer of the cerebellar cortex in rats was studied after blockage of potential-dependent Na^+ conductance with tetrodotoxin (TTX, 10^{-5}) and potential-dependent K^+ conductance with 3-aminopyridine (3-AmP, 5 x 10^{-3} M). The drugs were applied by superfusion utilizing a push-pull cannula kept in place by an agar dome which also served to stabilize the cerebellum. Following the application of the above drugs, no sign of field potentials generated by either local or white matter stimulation could be evoked. Thus, the parallel fiber field potential and the antidromic and orthodromic activation of the surface of the cerebellar cortex was absent.

As previously reported in pigeons (Llinás, R. and Hess, R.: *Proc. Natl. Acad. Sci. 73*, 2520-2523, 1976), intra- and extracellular recordings after TTX and 3-AmP indicate that the dendrites of Purkinje cells are capable of generating action potentials following either extracellular or direct electrical stimulation. In addition, activation could be obtained by extracellular iontophoretic injection of sodium glutamate from an electrode containing a 1M solution. Their overall properties were enhanced by increasing the Ca^{++} concentration in the superfusate to 2.5 mM. Finally, superfusion with $MnCl_2$ (10mM), which itself does not block either parallel fiber activity or the antidromic invasion of Purkinje cells, was shown to block the TTX— 3-AmP resistant dendritic electroresponsiveness. It is concluded that Purkinje cells in mammals can generate Ca^{++} spikes. Their possible role in functional modifiability will be discussed. (Supported by USPHS grant NS-13742 from NINCDS)

171 A DETAILED STUDY OF CLIMBING FIBER PROJECTIONS TO THE ANTERIOR FOLIA OF THE PARAMEDIAN LOBULE OF THE CEREBELLUM IN THE CAT. J.P. Lund, T.S. Miles* and J. Courville. Fac. Méd. Dent. and Centre Rech. Sci. Neurol., Univ. de Montréal, Canada.

Previous experiments have compared the somatotopy of cortical and peripheral inputs to the cerebellar cortex via climbing fibers (CF). The majority of single CFs in the anterior lobe respond to both inputs (Allen et al., Exp. Brain Res. 20: 255, 1974; Miles and Wiesendanger, J. Physiol. 245: 425, 1975), but a number appears to receive only a peripheral projection. In view of the fine somatotopic distribution of cerebro-cerebellar projections, this might be explained by an improperly located cortical stimulus. Alternatively, 2 or more populations of CFs to a given region might exist, some responding to the periphery alone, others to convergent inputs. In the present study, the analysis of the fine topography of cortical and peripheral CF inputs to a restricted region, the anterior folia of the paramedian lobule, was carried out in paralysed, nembutal-anesthetized cats. Penetrations were made at 200 μ intervals across folia a and b and the peripheral receptive fields of CFs were mapped. Responses to stimulation of the infra-orbital (I.O.) superficial radial (S.R.N.) and sciatic nerves of both sides were recorded as were those to stimulation of each of an array of 8 electrodes inserted into the pericruciate somatomotor cortex. CF responses to stimulation of one or more cortical sites were elicited in 96% of all Purkinje cells tested: 65% of those also received an input from the periphery, but 31% did not. Only 4% could not be excited from the cortex, probably because no electrode was placed in the region projecting to them. The majority of neurones had small tactile receptive fields on the ipsilateral forelimb. The hindlimb was also represented. CFs responded to stimulation of peripheral nerves at a characteristic latency. The majority fired at short latency (16-34 msec), others fired at short and long latency (120-250 msec) and a few, only at long latency. Those firing at long latency rarely had a peripheral receptive field. A clear correlation was observed between the cortical and peripheral sites projecting through a CF. 78% of all CFs had a cortical best point which received an input from the same region of the periphery from which the CF was also excited. It would appear that all CFs to this region of the cerebellum receive an input from the somatomotor cortex. When present, the sensory receptive field of these CFs is on that part of the body which would move in response to a motor command from the same region of cortex.

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172 THE NUCLEUS OF THE PONTOBULBAR BODY. A SEPARATE PRECEREBELLAR NUCLEUS? George F. Martin and J.M. Walker, Depts. of Anat. and Psychol., The Ohio State Univ., Columbus, Ohio, 43210.

The nucleus of the pontobulbar body (PBu) is situated along the migration route of neurons from the rhombic lip of the fourth ventricle to the basilar pons. Partly because the basilar pons is formed from that migration, the PBu is often included as part of the pontine grey.

It appears from material in our laboratory, however, that the opossum PBu is not simply a remnant of the pontobulbar migration, but that it has afferent connections which are highly organized and different from those of the basilar pons proper. Although the PBu has an input from the face motor-sensory cortex, like the pontine nuclei, it also receives projections from the red nucleus and spinal cord, regions which generally are not considered as sources for afferent projections to the pons. The PBu also receives a small input from the cerebellum. There is overlap of these projections within the PBu, although they each tend to have their own sphere of influence. A parallel finding is that PBu neurons contain reaction product after horseradish peroxidase injections into many areas of the cerebellar cortex. Although there is some topography to the PBu-cerebellar projection, it is not sharply defined.

Taken together such evidence suggests that, in the opossum at least, the PBu is as different from the basilar pons as either the reticulotegmental or lateral reticular nuclei and that it deserves to be set off from the basilar pons as a precerebellar cell station in its own right. Certainly its position along the pontobulbar migration should not be used as evidence against such a designation.

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173 OPERANTLY CONDITIONED SACCADIC EYE MOVEMENTS AND CEREBELLAR UNIT ACTIVITY IN THE CAT. J.G. McElligott and L.E. Mays. Dept. of Pharm., Temple Univ. Medical School, Phila., Pa. 19140.

Cerebellar neuronal activity from the posterior vermis (lobes VI and VII) was recorded in 2 cats that were trained to make precise saccadic eye movements. These animals were fitted with an ocular magnetic search coil in order to record horizontal and vertical eye movements, and absolute eye position. With it's head restrained, each cat was placed 70 cm in front of a square matrix array (10 x 10) of green light emitting diodes mounted on an opaque black board. Each trial was initiated by the sound of a buzzer. A center light came on and the animals were trained to fixate on it for 0.75 sec. Subsequently, a second light appeared randomly in any of the four directions (up, down, right or left). The animals were required to make a saccadic eye movement to this second light and fixate on it for 0.75 sec in order to obtain a milk reward. Thus, the task allowed us to dictate absolute start position, amplitude, and direction of the saccade. Cats were trained to generate from 200 to 300 correct responses per daily session. They would produce saccades with an average latency of 180 msec and an accuracy of about 1°. Forty-two cells were comprehensively analyzed with respect to sensory (auditory and visual) and oculomotor aspects of this task. Twenty-six percent of these neurons produced increases or decreases (latency=40 msec) in background activity that were related to sensory stimulation. The vast majority of these sensory related neurons (82%, n=9) produced increases in firing rates (duration=50 msec) after auditory stimulation (buzzer sound). Saccadic related neurons (24%, n=10) were intermingled with these sensory related neurons. These produced increases and/or decreases in background activity that were either related to one or more than one direction of movement. Furthermore, these short phasic changes could occur either before, during or after the initiation of a saccade. This is in agreement with our previously reported results for spontaneous saccadic eye movements (Soc. Neurosci. 6 #174, 1976). In addition, half of these eye movement related neurons manifested changes in tonic firing rates (increases or decreases) that were related to eye position during fixation. Finally, a small percentage of these neurons (5%, n=2) are related to both sensory and motor aspects of the task.

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174 THE INFERIOR OLIVARY COMPLEX IN THE RAT: GROSS NUCLEAR ORGANIZATION AND TOPOGRAPHY OF OLIVOCEREBELLAR PROJECTIONS. M. K. McGrane*, D. J. Woodward, M. A. Eriksson, R. A. Burne, and J. A. Saint-Cyr. Dept. Cell Bio., Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235.

This study was carried out to evaluate the topographic organization in the rat of cerebellar afferent information relayed via the inferior olivary complex to the cerebellum. Examination of a detailed series of coronal sections stained with routine cytological methods, the use of retrograde transport of horseradish peroxidase (HRP) and antrograde transport of tritiated amino acids are being employed in this investigation.

Initial studies were undertaken to determine the anatomical interrelationships of the cell groups constituting the inferior olivary nucleus (IO) of the rat. Serial coronal sections of IO stained with cresyl echt violet were examined by light and dark field microscopy and sections drawn by means of camera lucida. Three major discrete olivary subnuclei were noted including: 1) the principle olivary nucleus (PO) with a dorsal and a ventral lamella (DL and VL); 2) the medial accessory olivary nucleus (MAO), and 3) the dorsal accessory olivary nucleus (DAO). Five minor subnuclei were noted including the β nucleus (β), the dorsomedial cell column (DMCC) of the MAO, the ventrolateral outgrowth of Kooy (VLO), the dorsal cap of Kooy (DC), and a dorsal cell column (DCC) dorsally and medially to the medial accessory olive. Two unusual observations were: 1) just caudal to the dorsal and ventral lamellae the ventrolateral aspects of the DAO and MAO were found to fuse near the ventral surface of the medulla; and 2) the DAO at its most caudal extent folds over on itself dorsally and medially at its lateral edge to form what we designate as the "dorsal fold". The bulk of the olivary complex can be conceived of as a continuous sheet consisting at various levels of five distinct layers. The MAO is continuous medially with the VL; the VL laterally with the DL; the DL dorsomedially with the DAO, and the DAO laterally with the dorsal fold of the DAO at its caudal extent.

An evaluation of the localization of olivocerebellar projections by HRP injections into various cerebellar lobules indicated the following: 1) the lateral lobules of the cerebellum receive fibers from the rostral half of the PO, MAO, and DAO; 2) the anterior cerebellar vermis receives projections from the caudolateral regions of the MAO and the dorsal fold of the DAO; while 3) posterior vermis receives from the caudomedial MAO and β nucleus. The gross organization is that rostral and caudal olive project to lateral and midline cerebellum, respectively, but with specific olivary zones highly related to discrete areas in the cerebellum.

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175 IN VITRO STUDIES OF INTERACTIONS AND SURVIVAL OF CEREBELLAR CELLS FROM STAGGERER VS. WILD-TYPE MICE. Anne Messer and Mary E. Hatten,* Dept. Neuropathology, Harvard Medical School, Boston, Ma. 02115

A distinction must be made between genetic factors intrinsic and extrinsic to a specific degenerating cell type if neurological mutants that show such effects are to be used to assess cause-and-effect correlations of neural development. When the growth of granule cells from cerebella of staggerer (sg/sg) mutant mice is investigated in monolayer cell cultures using modified Hams F12 medium plus fetal calf serum, cells from the mutant are found to clump less and survive longer than their wild-type counterparts. Thus, the degeneration of granule cells observed in these mutants in vivo cannot be a function of irreversibly programmed cell death before postnatal day 7, the age at which cells are dissociated (Messer & Smith, Brain Res., in press).

The possibility that the increased survival is a function of cell-cell interactions is examined in two systems. In one, normal cells grown on glass, plastic, or polylysine-coated glass are compared to each other, and then the behavior of sg/sg vs. control cells are compared under the same conditions. Although the polylysine coating both reduces the amount of initial clumping and increases the survival of normal cells, it does not completely eliminate the difference between mutant and control. Mutant and control cultures exhibit the same behavior only when culture conditions are changed to include supplementation with horse serum instead of fetal calf serum in addition to the substrate coating.

A second, more direct test of cell surface differences, uses the agglutinins Concanavalin A, ricinus communus and wheat germ agglutinin (WGA). It shows that while WGA causes dissociated postnatal day 7 sg/sg cells to agglutinate, there is no such effect on control cells using any of these lectins, or on mutant cells with the first two. Thus, there must be some specific cell surface differences between sg/sg cerebellar cells and their controls.

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176 THE BASILAR PONTINE GRAY IN THE RAT: AN INITIAL LOOK AT ITS CYTOLOGY AND SYNAPTIC ORGANIZATION. Gregory A. Mihailoff. Dept. Cell Biology, Univ. of Texas Hlth. Sci. Ctr. at Dallas, Dallas, Texas 75235.

Cytoarchitectonic analysis of Nissl-stained, transverse sections of the basilar pontine nuclei in the adult rat reveals the presence of five major divisions named with respect to the longitudinally directed fiber bundles comprising the cerebral peduncle. These include medial, ventral, lateral, dorsal, and peduncular nuclei. An apparent increase in cell-packing density as compared to the opossum (Mihailoff and King, J. Comp. Neur. 159: 521-552, 1975) and cat (Brodal and Jansen, J. Comp. Neur. 84: 31-118, 1946) may account for the difficulty in defining certain other smaller subnuclei such as the median and dorsolateral groups reported in the aforementioned studies in the opossum and cat.

Observations on the form of individual neurons in the pontine gray of the adult rat as demonstrated in Golgi-Kopsch prepared material suggest the presence of two general classes of cells; a projection neuron whose axon presumably terminates in the cerebellar cortex as a mossy fiber and an intrinsic cell whose processes ramify exclusively within the pontine gray. These results are in agreement with the previously cited study in the opossum as well as those in the mouse (Cajal, Histologie du Systeme Nerveux, p. 962, 1906). One interesting difference, however, is that in the rat, characteristics such as numbers of dendritic spines, the frequency of dendritic branching and the length of individual dendrites are quite variable within the general category of projection cells and seem to justify the designation of at least two sub-populations of such neurons.

Electron microscopic examination of rat pontine gray neuropil in aldehyde perfused control brains suggests the presence of at least two types of neuronal somata, one variety exhibiting a smooth contoured nucleus, the other, a highly involuted nucleus. Three general categories of presynaptic profiles can be distinquished and include those containing either round, flattened or pleomorphic synaptic vesicles. The relationship of such vesicle-containing profiles to known basilar pontine afferents and intrinsic elements will be discussed in the context of their role in pontine gray synaptic organization.

177 RUBROBULBAR PROJECTIONS IN THE TREE SHREW (TUPAIA). Heather M. Murray. Dept. Anat., Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.

Radio-frequency lesions were stereotaxically placed in the red nucleus of 16 adult tree shrews. After 4-7 days, the animals were perfused with 10% formalin and brain tissue was stained for light microscopy by the Fink-Heimer method. Cresyl violet acetate was used to stain alternating serial sections for morphological analysis. Degenerating axons from lesions in the caudal two-thirds of the red nucleus were traced across the midline to the ventrolateral border of the contralateral brain stem. Fibers of passage and preterminal degeneration were distributed to the chief sensory nucleus of the trigeminal nerve, medial portions of the spinal nucleus of the trigeminal nerve, lateral areas of the facial nucleus and of the lateral reticular nucleus, and also to a small group of cells presumed to be group f of the inferior vestibular nucleus. Degeneration at the level of the lateral cuneate nucleus extended into areas presumed to be Groups X and Z, and ventrolateral areas of the cuneate and gracile nuclei. Ipsilateral to these lesions a few fibers of passage and a slight amount of preterminal debris was found in the facial nucleus. Lesions that involved the reticular formation surrounding the red nucleus produced relatively large amounts of degeneration in the pontine and medullary reticular formation and also within dorsomedial areas of the facial nucleus contralateral to the lesion. Ipsilaterally, these lesions produced only slight to moderate amounts of degeneration. Lesions that involved only the rostral one-quarter or rostral pole of the red nucleus gave rise to no degeneration in the areas receiving projections from the caudal two-thirds of the red nucleus. (Supported by GRS grant 5 S07 RR 05583-12).

178 NEURONAL AND GLIAL ELEMENTS OF CEREBELLUM VISUALIZED WITH NEW INTRACELLULAR STAIN. C. Nicholson, S. B. Kater, and W. Stewart*. Dept. Physiol. & Biophys., N.Y. Univ. Med. Ctr., New York, NY 10016; Dept. Zool., Univ. Iowa, Iowa City, IA 52242; and NIH, Bethesda, MD 20014

Intracellular staining, using stain ejected from a micropipette directly into a cell, has become a powerful tool for revealing cellular geometry. The most successful stains have been the reactive fluorescent dye, Procion yellow, the metal salt, cobaltous chloride and the protein, horseradish peroxidase. We report the use of a new fluorescent dye, Lucifer yellow, to stain elements of the rat cerebellum.

Rats were anesthetized with urethane and the cerebellum exposed. Micropipettes contained a 3% aqueous solution of Lucifer yellow CHL (resistance of 50-100 MΩ). When a cellular element was penetrated and showed a stable resting potential stain was injected with a negative current of 10-50 nA for 0.5-10 minutes. Some cells gave injury discharges on penetration, indicating that they were neurons, others had high negative (-70 to -80 mV) resting potentials and no discharge, suggestive of glia. At the end of the experiments, (duration about 4 hours), animals were perfused with a 4% solution of formaldehyde and frozen sections cut at 50-100 μm. Sections were dehydrated, mounted in Entellan and viewed under a fluorescent microscope (excitation filter, Schott BG 12, barrier filter 470 nm cutoff).

Purkinje cell dendrites and somata appeared to stain completely, including spines; axons were occasionally seen. Golgi and Basket cells were stained, including both dendrites and axonal plexi. The profiles of these three neurons resembled the classical Golgi stain except that Lucifer yellow gave a more delicate appearance. Numerous glial elements were visualized. Glia stained incompletely and seemed to be composed of many fine filamentous structures, differing from typical Golgi representations. Thus Lucifer yellow migrates more extensively in neurons as compared to glia.

We believe that Lucifer yellow is superior to Procion in its ability to more completely impregnate cellular elements; this may be due to its greatly increased visibility. Like Procion, however, Lucifer yellow will fade on prolonged exposure to a high intensity light source. Unlike cobalt, horseradish peroxidase or the classical Golgi stain, Lucifer yellow does not involve the formation of an intracellular precipitate and thus may yield a more accurate representation of fine structure. Supported by USPHS Grants NS-13742 (CN) NS-09696 and AM-19858 (SBK) and Natl. Inst. Arthritis, Metabolic and Digestive Diseases (WS).

179 PROCESSING OF EYE MOVEMENT SIGNALS IN MONKEY FLOCCULUS. Hiroharu Noda (SPON: S. Hagiwara). Brain Research Institute, Depts. Physiol. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

In the flocculus of the monkey, discharges of Purkinje cells (P-cells), interneurons, and afferent fibers were compared during saccadic and tracking eye movements, and during steady fixation. As previously reported, the typical behavior of P-cells during a saccade was complete cessation of firing, starting 10-15 msec prior to the saccade (1). During a period of silence in P-cells, interneurons showed a burst of spikes and mossy fiber units showed two types of behaviors. One class of fiber units started a burst 10-20 msec before a saccade, showing peak activity at the beginning of the saccade. The other class of fiber units showed a gradually increasing activity starting 100-200 msec before the saccade, and this long lasting burst reached peak activity at the beginning of the saccade.

During steady eye position, the discharge rate of some P-cells was clearly a linear function of eye position (1). This type of linear relationship was also found in some interneurons and mossy fibers. However, in most units this linearity was limited to only a part of the range of eye positions, and the firing either completely disappeared or reached a maximal rate when the eyes shifted outside this part of the total range. These differences in activity between P-cells and their input cells indicate the manner by which eye movement signals are processed within the flocculus. (Supported by NIH Grant EY01051)

(1) H. Noda and R. Asoh, Neuroscience Abstracts 2, 1976, P115.

180 ANALYSIS OF ANURAN VESTIBULO-CEREBELLAR CONTROL BY COMPUTER MODELING. A. Pellionisz and R. Llinas. Dept. Physiol. & Biophys., N. Y. Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

A software computer model was developed as a self-consistent framework to study global functional properties of the vestibulocerebellar system of frog. The model was based on existing morphological and physiological data from the vestibular system and the cerebellum. For the latter the neuronal circuitry, as well as the particular functional properties of cells, was implemented. The actual detailed activity of given cells was simulated by means of a multicompartmental model using Hodgkin-Huxley parameters for each compartment. A previous study (Pellionisz, Llinas & Perkel: *Neuroscience 2:* 19-35, 1977) modeled the anuran cerebellar cortex and gave us a first order description of the spatial distribution of neuronal activity produced by inputs through particular sets of mossy fibers

In the present study the physiological activation of the peripheral vestibular system was simulated. This input was projected onto a model vestibular nucleus and cerebellar cortex. At the cortex the spatial organization of the Purkinje cells activated by different kinds of rotation (pitch, roll and yaw) was displayed. The overall distribution of the activity in these modeled neurons suggests that much of the functional specificity found in this cortex does not require specific connectivity. Rather, it seems to be related to the location of the mossy fiber input with respect to the granular layer and in particular with its location in the cerebellar peduncle. These properties, such as spatial distribution of Purkinje cell thresholds and dynamic responsiveness of these cells to tonic and phasic inputs, were displayed and analyzed by the model. The study suggests that such patterns of activity may in fact represent emergent properties inherent in the morphological organization of the circuit. The output of the cortex was utilized as a closed loop control system in the modulation of limb movement. This type of modeling must be considered, therefore, as an heuristic tool which can provide an independent test for many of the neuronal circuit hypotheses which are constantly being postulated in neurobiology. (Supported by USPHS grant NS-13742 from NINCDS)

181 ACTIVITY RELATED TO THE ELECTRIC ORGAN DISCHARGE IN THE CEREBELLUM OF A MORMYRID FISH. C. J. Russell and C. C. Bell, Neurological Sciences Institute, Good Samaritan Hospital, Portland, Oregon 97210.

Extracellular recording throughout the valvula cerebelli of Gnathonemus petersii reveals occasional afferents which fire in relation to the electric organ discharge command signal. But in most regions, units in the valvular ridge (Purkinje cell area) only rarely exhibit strong relationships to this signal. In one area, however, the discharge of afferents in relation to the command is very prominent, indicating strong and synchronous activation. Furthermore, unitary and other activity in these valvular ridges is strongly related both to the command and to electrical stimulation of the electroreceptor surface. This area appears to be quite restricted, and possibly comprises only one or two ridges in the ventral portion of the valvula.

The afferent volley following the command occurs about 15-20 msec after the command signal recorded from the tail in curarized preparations. The volley is accompanied by a field potential which is negative with respect to the dorsal surface of the valvula. This potential reverses polarity as the recording electrode is moved from the granule cell area into the ridge. Units in the ridge have been observed to be excited at the time of the afferent volley, inhibited at the time of the volley, inhibited for a period following the volley, or excited for a period following the volley. A type of activity which is highly variable in waveform, with predominantly low frequency components, occurs from 40-80 msec following the command signal. This activity is reminiscent of dendritic spikes or of climbing fiber activity.

Units respond to electrical stimulation of the skin with thresholds similar to those of Mormyromast receptors. When such stimuli are given during the time when electroreceptors would normally be activated by the electric organ discharge the responses interact with the command-related activity. This interaction has been observed to take the form of inhibition, excitation, or change in the time course of the response. The time course of the responses to both command and to external stimuli, the response thresholds to external stimuli, and the nature of the interactions all suggest that this area of the valvula is related to the processing of electrosensory information arriving via Mormyromast afferents and is thus related to electrolocation. The fact that the area in which these responses are found is so restricted indicates that the valvula is also functionally quite diverse. (Supported by NIH (NINCDS-06728) and NSF (BMS73-06867))

182 MICROGEOMETRY OF CEREBELLAR CORTICONUCLEAR PROJECTION IN THE PIGEON. Dietrich W.F. Schwarz and Stuart Wood*. Depts. Otolaryngology and Anatomy, Univ. of Toronto Toronto, Ontario, Canada.

Previous evidence indicates that Purkinje cells (PC) within longitudinal sagitally oriented stripes of cerebellar cortex project onto distinct cerebellar nuclear regions. If this pattern were to hold for the geometrical arrangement of PC projection onto single nuclear cells it would be difficult to conceive how the information mediated by parallel fibers through transversally oriented rows of PC could be utilized at postcerebellar levels. We studied the geometrical layout of the PC projection to small nuclear zones. Small horseradish peroxidase injections into the cerebellar nuclei of pigeons were achieved by means of an electrophoretic deposition technique via recording microelectrodes. As a result relatively small numbers (50-450) of PC were found labelled in serial sagittal frozen sections of the cerebellum. The location of these PC was precisely charted on maps which represent the cerebellum as one flat sheet of tissue with the folia imagined unfolded. A strong tendency for labelled PC to be arrayed transversally, i.e. in the direction of parallel fibers, is evident on these maps as shown on our poster. It is concluded that the known longitudinal arrangement of the cortico-nuclear projection merely represents a gross anatomical structurization, whereas the finer convergence pattern is organized transversally.

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183 FRACTURED SOMATOTOPY OF MINIATURIZED PATCH-LIKE MOSAICS IN GRANULE CELL TACTILE AREAS OF RAT POSTERIOR CEREBELLUM AS REVEALED BY MICROMAPPING. Georgia M. Shambes, Jon W. Joseph*, John M. Gibson, and Wally Welker. Department of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

We have discovered somatic sensory receiving areas in the caudal cerebellum previously unknown in any mammal. Patterns of tactile projections to granule cell (GC) layer in cortex of posterior cerebellum (hemispheres and vermis) in 40 anesthetized albino rats were defined using tungsten micromapping techniques. We found four distinct relatively short latency GC tactile areas. Multiple unit responses were evoked by threshold natural stimulation of cutaneous tissues. Data were obtained from 1430 recording locations. Peripheral projections terminate within columnar assemblies of granule cells. These columnar inputs appear as individuated patches or tiles of different sizes and shapes with major axes ranging from less than 500 μm up to 1500 μm. The overall array of adjacent columnar patches in each area collectively make up a mosaic. Somatotopic organization within each single patch is highly specific, whereas somatotopy is fractured or disjunctive from patch to patch within the mosaic. Electrode densities as high as 75/mm^2 were required to delineate the fine grained features of the projections within patches. Each patch of the mosaic receives input from a definable type of cutaneous structure or region. The fact that adjacent patches receive inputs from body structures that often are used together in discriminative or instinctive behaviors suggests that particular patterns of contiguity of the patches within a mosaic may constitute a neural substrate for such act sequences. Our data support Snider's early contention that tactile projections, rather than proprioceptive inputs alone, play an important role in cerebellar sensori-motor functions. These findings necessitate re-examination of several current views regarding afferent, efferent and intra-cerebellar circuits and their functions. (Supported by NSF grant BMS 75-08124 and by USPHS grants NS06225 and NS07026.)

184 VISUAL MESSAGE UNITS OF THE RABBIT CEREBELLAR FLOCCULUS. John I. Simpson and Rainer Hess, Dept. of Physiol. & Biophys., NYU Med. Sch., N.Y., N.Y. 10016 and Neurobiol. Sec., Max-Planck-Inst. for Biophys. Chem., Goettingen, W. Germany.

Previous investigation of the visual messages of the cerebellar flocculus has focused on the climbing fiber (CF) input evoked from the ipsilateral eye. We now report on some of the visual responses of other types of neuronal elements in the flocculus. Experiments were performed in anesthetized, immobilized rabbits. Extracellular unit recordings were made primarily from that portion of the flocculus which receives both mossy fiber and CF visual inputs. Visual stimuli consisted of a large field Julesz pattern moving with various velocity step profiles. Purkinje cell CF activity modulated by stimuli presented to the contralateral eye showed direction and speed selectivity. Optimal speed was about 1°/sec., as found previously for CF's driven from the ipsilateral eye. Contralaterally driven CF's were most strongly activated by movement in a direction ± 30° from vertically downward. This preferred direction differs from those found for ipsilaterally driven CF's. In cases where both CF and simple spike (SS) responses of a Purkinje cell were modulated by stimuli presented to one eye, the changes in their respective activity were oppositely directed, but with different time courses. In response to a velocity step of a few degrees/sec. the change in CF activity consisted of an initial transient overshoot (peak latency about 0.5 sec.) followed by a return to a plateau level. In contrast, the SS activity gradually changed over 1-2 sec. to reach a plateau level. Modulation of the Purkinje cell SS response in opposite direction to that of the CF response could be found in floccular areas in which only CF field potentials were evoked by flash stimuli. This observation is consistent with the disfacilitatory effect on Purkinje cells of the CF collateral projection to Golgi cells. The reduction in SS activity associated with an increase in CF activity was not manifested as a specific silent period following each CF response. Integration and differentiation of the velocity profile of the visual stimuli are reflected in the response profile of some floccular units. An example of a (saturated) integration is present in response profiles showing that the rate of change of firing frequency but not the final plateau level is related to the stimulus speed. An example of a (double) differentiation is present in response profiles showing that the 'edge' of a velocity step is enhanced by an activity pattern similar in form to a Mach band. A question of interest is to what extent are these integral and differential operations dependent upon cerebellar cortical circuitry? (Supported by USPHS grant NS-13742 from NINCDS).

185 TOPOGRAPHICAL ORGANIZATION OF DENTATE AND INTERPOSITUS PROJECTIONS TO THE THALAMUS IN DOG AND CAT. G. B. Stanton.* (Sponsor: Duke Tanaka, Jr.), Department of Anatomy, College of Medicine, Howard University, Washington, D. C. (20059).

Studies of cerebellofugal fibers in the monkey have indicated a topographical organization of projections to the thalamus from the dentate and interpositus anterior nuclei with a distinct difference in the mediolateral distribution of terminals from each nucleus (Stanton, '77). The results of the present study suggest that the dentatothalamic and interpositothalamic projections in the dog and cat display a similar organization. Discrete lesions and/or localized isotope injections were placed in the dentate and interpositus nuclei of the dog and cat. In some cases these were combined with horseradish peroxidase injections in the ipsilateral motor cortex. Degenerating axons and axon terminals were stained with a modified Wiitanen technique. Lesions of the dentate nucleus in the dog produce 3-5 patches of preterminal degeneration paralleling the internal medullary lamina in the medial part of VL. Ventral parts of the dentate project heavily to the dorsal arch of the VA-VL complex and the dorsal parts project mainly to centrolmedial regions of VL. These results suggest an inverted projection from the dors oventral axis of the dentate to the thalamus as is the case in the monkey. Projections from the interpositus anterior to the thalamus in the dog are less abundant than those from the dentate and more restricted in the anterior-posterior plane. Interpositus anterior projections terminated ventral and lateral to the projections from the dentate in the middle third of VL. The patchy distribution of interpositus terminals in the middle third of VL was similar in the cat as seen with autoradiographic techniques. These results will be related to a possible somatotopic organization of the dentate and interposed nuclei. This investigation was supported by the General Research Support Grant No. 5 S01 RR 05361 from the General Research Support Branch, Division of Research Resources, National Institutes of Health.

186 STAGGERER MUTANT MICE AND NORMAL LITTERMATES EXPRESS DIFFERENT CARBOHYDRATES ON POSTNATAL CEREBELLAR CELLS. E. Trenkner*, K. Herrup, M. E. Hatten*, and S. Sarkar. Dept. Neuropath., Harvard Med. Sch., Boston, Ma. 02115, Div. Med. Genetics, Dept. Med. M-013, Sch. Med. UCSD, La Jolla, Ca. 92093. Several anti-carbohydrate antibodies have been shown to inhibit reversibly nervefiber outgrowth by early postnatal and embryonic cerebellar cells in microwell cultures (1, 2), demonstrating that embryonic and postnatal tissue expresses different carbohydrate moieties. In the current study an anti 1 6 mannan antibody (against yeast) and an anti sialic acid antibody (against Neisseria meningitidis type B) (2) were used to probe cell surface carbohydrates of cerebellar cells from C57B/6 staggerer (sg) mutant mice as compared with their normal littermates. Anti 1-- 6 mannan antibody inhibited in vitro fiber outgrowth between reaggregated clusters of cells from normal 7 day old (P7) cerebellum, but not by cells from P7 sg cerebellum. In cryostat sections, FITC-conjugated anti 1-- 6 mannan antibody stained granule cells of both external and internal granule layers and Purkinje cell bodies of normal P7 cerebellum, but no staining was observed in sections of P7 sg cerbellum. Anti 1-- 6 mannan antibody, therefore, recognizes a mannoside-containing component of normal but not sg cerebellum. Anti sialic acid antibody gave a different reaction. No inhibition of in vitro fiber outgrowth in microwell cultures was observed with P7 cerebellar cells treated with this antiserum (2). However, fiber outgrowth was inhibited in microwell cultures of both embryonic day 13 normal cerebellar cells and P7 sg cells. In addition, whereas postnatal days 13 to 15 cryostat section of normal cerebellum were not labeled with FITC-conjugated anti sialic acid antibody, cryostat sections from postnatal day 13-15 sg cerebellum were heavily labelled. Both the inhibition of fiber outgrowth in vitro and the fluorescent labelling in cryostat sections was blocked specifically by N. meningitidis type B polysaccharide (a sialic acid polymer). The anti sialic antibody, therefore, appears to probe a sialic acid-containing moiety present on normal embryonic and postnatal sg cerebellar cells, but not on normal postnatal cells. These results are in agreement with similar studies performed with plant lectins (3) and suggest that postnatal staggerer cerebellar cells differ from normal cells of the same age with respect to at least two cell surface characteristics.

1. Trenkner, E., and Sidman, R. L. (1977) submitted.
2. Trenkner, E., and Sarkar, S. (1977) J Supramol. Struct. Suppl. 1, p. 25
3. Messer, A., and Hatten, M. E. (1977) Neuroscience Abstr

CEREBRAL CORTEX

187 LONG RANGE ORDER IN CEREBRAL MACROMOLECULES SUGGESTED BY ELF, VHF and UHF FIELD EFFECTS ON CALCIUM BINDING. W. Ross Adey and Suzanne M. Bawin*. Brain Res. Inst., Sch. Med., UCLA, Los Angeles, CA 90024.

Chemical interactions at specific amplitudes and frequencies of weak imposed electromagnetic fields may arise in cooperative processes involving long range order between charge sites on transductive macromolecules at membrane surfaces. In a search for long range order in anionic binding sites on cell surface macromolecules, we have used ELF fields in the range 1 to 100 Hz, VHF fields at 147 MHz amplitude modulated at frequencies from 1 to 30 Hz, and 450 MHz UHF fields also amplitude modulated at frequencies from 1 to 30 Hz. We have found a series of amplitude and frequency "windows" that strongly suggest resonant interactions based on long range order. ELF fields between 6 and 20 Hz reduced $^{45}Ca^{2+}$ efflux from chick and cat forebrain tissue by about 15%, with a maximum effect for fields in air at 10 and 56 V/m. Effects were insignificant at 5 and 100 V/m. Isolated chick cerebral tissue was also exposed to a 147 MHz field, 0.8 mw/cm^2 and amplitude modulated at frequencies from 0.5 to 35 Hz. There was a frequency "window" for increased efflux (15%) at modulating frequencies from 9 to 20 Hz. We have varied the intensity of a 450 MHz field, amplitude modulated at 16 Hz, from 0.1 to 5 mw/cm^2. There was an amplitude "window" for increased calcium efflux between 0.375 and 1.0 mw/cm^2. We hypothesize that membrane surface charge sites behave "coherently" over a considerable area, and that a coherent patch may be triggered to change state by a very weak trigger at one point. This trigger event may involve proton tunneling at the boundary of such a patch.

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188 OBSERVATIONS ON PYRAMIDAL CELLS OF REGIONS CA3 AND CA4 IN THE HIPPOCAMPUS OF REELER MICE. T.V.P. Bliss*, M.L. Errington* and R. Victoria Stirling*. (SPON: J. Dostrovsky). Nat. Inst. Med. Res., London, NW7 1AA.

The morphology, ultrastructure and pattern of afferent and efferent connections of pyramidal cells in regions homologous to CA3 and CA4 of the normal mouse have been examined in reeler littermates.

Morphology. Observations on Golgi impregnated material show that CA3 cells in reeler have a roughly normal pattern of dendritic arborization despite abnormalities in cell position. CA4 cells in reeler are widely scattered among the granule cells which occupy the hilar region, as shown in Golgi material and by retrograde transport of HRP injected into the contralateral hippocampus. Dendrites of CA4 cells which in the normal animal are confined to the hilus of the dentate gyrus, in reeler assume a stellate configuration with dendrites extending into the granular layer, and in some cases penetrating through the molecular layer to the pial surface. The dendritic excrescences receiving mossy fibre terminations in both CA3 and CA4 pyramids are limited in both reeler and normal animals to the proximal third of the dendritic tree.

Ultrastructure. In the hilar region of both reelers and normal littermates characteristic mossy fibre synaptic profiles are seen in the EM. In reeler, mossy fibre terminals have also been found in the supragranular part of the molecular layer.

Distribution of afferent and efferent fibres. The terminal field of the mossy fibre input to CA3 and CA4 cells was visualised using the Timm's staining method. In reeler, the densely staining mossy fibre band is expanded to accomodate the spread of CA3 cells. In Golgi material, mossy fibres in reeler mice are sometimes seen meandering in the supragranular region before exiting from the hilus. These axons and their terminals are visible in Timm's sections as dense supragranular clumps. Commissural projections were studied using the HRP technique. Injections of HRP into subfields CA3 or CA4 of both normal and reeler mice resulted in retrograde transport to pyramidal cells in contralateral CA3 and CA4 respectively.

These observations demonstrate that pyramidal cells in reeler mice, despite their abnormal locations, are interconnected via the commissural system in a normal manner. The results also emphasize that the position of target cells is a determining factor in the location of mossy fibre terminals.

189 MORPHOMETRIC AND FUNCTIONAL STUDIES ON EFFECTS OF 200 RADS CO-60 PRENATAL RADIATION ON THE BRAIN AND BEHAVIOR OF SQUIRREL MONKEY OFFSPRING. Kenneth R. Brizzee, Neurobiology Department, Delta Primate Center, Covington, Louisiana 70433.

Abnormalities of the brain represent frequently cited radiation effects after prenatal exposure. Species differences in biological dose-response effects indicate that differential radiation effects on various regions of the brain are most likely verifiable in diurnal primates since they are of the same taxonomic order as man and encephalization of brain functions is comparable. The aims of these morphological and functional studies were to compare offspring prenatally exposed to 200 rads Cobalt-60 radiation with 6 matched controls on days 1 and 2 after birth on: 1) brain weight, cortical depth, neuron-glia populations and dendritic spines; 2) neuromuscular reflexes and behavior; 3) homeostatic regulation of temperature, respiration and heart rate.

Prenatal exposure to 200 rads Cobalt-60 resulted in a significant reduction in body weight, crown-rump length and head circumference in the 2-day old offspring. There was also a significant reduction in brain weight and cortical depth in the motor cortex. Examination of cortical areas indicated a significant change in neuron and glia cell populations. Examination of dendritic patterns in cortical regions indicated significant reductions in dendritic spine counts. Regarding neurological and behavioral tests, significant prenatal radiation effects were observed in righting, head-up orientation and tail hanging reflexes, and on climbing performance on an inclined plane. Prenatal radiation exposure resulted in a significant increase in the basal and activation range in homeostatic regulation of temperature, respiration and heart rate. From the morphometric and functional studies it was concluded that extrapolations concerning prenatal radiation effects on regional variations in brain vulnerability and behavioral impairments suspected in man are most likely verifiable in higher diurnal primates.
(Supported in part by NIH Grant 1 R01 HD09942)

190 EFFECTS OF IMPLANTATION WITH VARIOUS TYPES OF SUBDURAL ELECTRODE ARRAYS. L.A.Bullara*, D.Jacques, R.H.Pudenz*, T.G.H.Yuen* and W.F.Agnew. Huntington Institute of Applied Medical Research, Pasadena, California.

The generally accepted procedure to fabricate electrode arrays for surface cortical stimulation is to imbed the electrodes in a silastic backing. This technique invariably leads to depression of the brain beneath the electrodes and the herniation of the brain around the periphery of the silastic disc. Extensive observations done at this and other laboratories consistently show this depression and herniation. Attempts to modify the silastic imbedding by using a thicker silastic backing to prevent buckling or thinner backing to allow conformation to the cortical surface have been unsuccessful. However, if the silastic material is eliminated altogether by supporting the electrodes on 9-0 nylon spokes significant reduction of the cortical depression and herniation have been observed. Supported by NINCDS contract No. NOS-NS-0-2275.

191 THE "CALLOSAL ZONE" IN THE FIRST AND SECOND SOMATOSENSORY AREAS OF THE CAT. Roberto Caminiti*, Giorgio M. Innocenti and Tullio Manzoni*. Institut d'Anatomie, Université de Lausanne, 1011 Lausanne, Switzerland, and Istituto di Fisiologia Umana, Università d'Ancona, Italy.

The regions of origin of callosal fibres (callosal zones cf. ref. 1) in the body representations in SI and SII of the cat were studied by retrograde transport of horseradish peroxidase (HRP). Two types of experiments were performed: a) Small, localized injections of HRP into SII, preceded by electrophysiological identification of the cutaneous district represented at the injection site. b) Multiple injections of HRP into areas SI and SII; in the same animals, in contralateral SI and SII, the position of HRP filled neurones (callosal neurones) relative to the somatotopic map was determined by electrophysiological recording of single units and electrode track reconstruction. For histological methods and data analysis see (2). It was found that the callosal zone in SI is discontinuous. A small, rather densely packed group of callosal neurones exists in the most anterior part of SI at the frontal end of the coronal sulcus (area 3a). Moving posteriorly along the coronal gyrus and up along the posterior sigmoid gyrus no callosal neurones are found until the middle portion of the latter gyrus. In SII callosal neurones are more widespread than in SI although their packing density is non homogeneous. In the anterior part of SI and in SII, callosal neurones are found in regions which contain a detailed representation of the distal part of contralateral forelimb. These neurones project to a part of SII where the distal part of the forelimb is represented. In both SI and SII most callosal neurones are in layer III, some in layers V and VI. In coronal sections, callosal neurones in SII appear arranged in clusters of irregular shape and distribution. Most callosal neurones are pyramidal; some are fusiform cells (layer VI) whose main dendrite can sometimes be traced to layer I; very few others are stellate cells. In layer III the average size of callosal neurones is significantly larger than that of neighbouring non callosal neurones. The extent and location of the callosal zones in SI and SII suggest that a transformation of the cortical somatosensory map takes place in the callosal message originating in these areas. The cortical regions of distal forelimb representation are not mute to the contralateral hemisphere.

1) Innocenti G.M., et al., Neurosc. Letters, 4 (1977) 237-242.
2) Innocenti G.M., and L. Fiore, Neurosc. Letters, 2 (1976) 245-252.

192 BEHAVIORAL CORRELATES OF NEURONS IN RAT SENSORIMOTOR CORTEX. John K. Chapin and Donald J. Woodward. Univ. Tx. Health Sci. Cntr., Dallas, Texas 75235.

It is known in the rat that there is a considerable overlap between motor cortex (defined as the regions with lowest threshold for movement elicited by electrical stimulation) and somatosensory cortex (the regions which give unit or evoked responses at the lowest threshold of peripheral stimulation). This study considered the question of whether neurons of the sensorimotor cortex (SmC) fire exclusively in relation to sensation, to movement, or to combinations of both in the context of ongoing behavior. Single unit recordings were obtained with microelectrodes driven into the SmC's of unrestrained, behaving rats chronically implanted with a movable microdrive apparatus. Each unit was observed for a period of one to six hours while the animal was (1) at rest; (2) oriented or vigilant to the approach of the investigator's hand; (3) mechanically stimulated in somatosensory receptive fields; (4) undergoing manipulation of its limbs by the investigator; and (5) exhibiting spontaneous exploratory movements or forced treadmill running. Preliminary studies of over seventy cells suggest three general categories of units. Most numerous (60%) were the small (200-400uV) units of all layers which possessed large, variable, and vaguely defined somatosensory receptive fields, were responsive to the approach of a hand during vigilance, but fired in marked correlation with general behavior states such as running, exploration and rest. Responsiveness of such cells to somatic stimulation typically attenuated during movement. The second most commonly found (25%) category included the large (400-1000uV) units of the middle and deep layers which possessed a low resting discharge but fired strongly under appropriate conditions. Some of these cells responded specifically to somatic touch, a few of which were driven only when the limb was held at a particular position. In the rostral part of the SmC most large cells fired in response to passive manipulation of limbs and also to active movement of those limbs. In states of whole body motion the firing relation to movement of specific parts of the body became less apparent. The least commonly found category included the medium size (300-500uV) units of the middle layers of the more caudal SmC which exhibited very regular, constant, and clearly delimited somatosensory receptive fields. These characteristics were preserved during movement. This data suggests the concept that somatosensory information is available to almost all SmC cells but is utilized differentially depending on neuron type and on the behavioral state of the animal.

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193 USE OF CORTICAL CIRCUITS DURING SEIZURES: AN AUTORADIOGRAPHIC STUDY WITH ^{14}C-DEOXYGLUCOSE. Robert C. Collins. Dept. Neurol., St. Louis, Mo. 63110

The injection of 25 units of penicillin into anterior motor cortex of rats in 0.1µl of CSF resulted in repetitive spike discharge for 90 minutes. ^{14}C-deoxyglucose autoradiography performed on animals with repetitive contralateral focal convulsions revealed a 2-3 x increase in glucose utilization in a distinct histological pattern in the cortex.

Immediately surrounding the injection there was metabolic activation of all cortical layers, with strict vertical borders on medial and lateral sides. The posterior third of the focus showed full activation of layers V & VI, with on-off columns extending into layer I. The focus ended in a single trailing column. Activation beyond the focus occurred ipsilaterally in 1-3 columns posterior and lateral to the focus in sensory cortex, and specific activation of Sm II above the rhinal fissure. The contralateral anterior motor cortex showed increased glucose utilization in deep layers (probably Vb & VI) with columnar patches in I-III. Throughout the cortex ipsilateral to the focus there was inhibition of normal activity in layer IV, and a more generalized depression of activity below control in all layers of cortex not specifically activated by the seizure.

Focal penicillin seizures result in an increase in glucose utilization in specific cortical circuits arranged in a columnar and laminar pattern, and in widespread decrease in glucose utilization in ipsilateral cortical areas around and beyond these circuits.

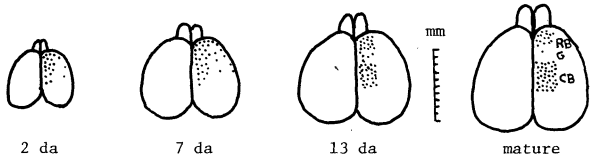
194 NORMAL AND POST-TRAUMATIC DEVELOPMENT OF CORTICOSPINAL NEURONS IN RAT STUDIED WITH HORSERADISH PEROXIDASE. Constance J. D'Amato and Samuel P. Hicks. Dept. Pathology, Univ. of Michigan Medical Center, Ann Arbor, MI 48109.

Corticospinal (CS) neurons in the mature rat labeled with HRP from their cut axons at cervical and lumbar cord levels are distributed in the dorsal cortex in a major caudal band, CB, and a minor rostral band, RB, separated by a gap, G, in which none or few neurons appear (see figures). CB overlaps Krieg's (1946) areas 3, 4, 6 and Hall's and Lindholm's (1974) forelimb to hindlimb motor areas. CS neurons labeled from cervical and lumbar levels are intermixed non-somatotopically in CB. RB, lying in area 10, can only be labeled from cervical cord (Hicks, D'Amato, 1977).

Labeling from cervical levels in rats 2, 4, 5, 7, 10, 13, 17, 21 days or later shows that CB and RB are continuous, the G regions containing many CS neurons, until about 13 days (see figures). After that they can no longer be labeled. Some CS neurons in the rostral medial cingular cortex as well as in cortex lateral to CB and RB can be labeled in the first week. The presence of neurons in G, medial, and lateral regions whose axons extend to the cervical cord only in infancy cannot be accounted for by the normal uneven growth of the cortex.

When the presumptive domain of CB is bilaterally ablated in early infancy, many CS neurons in the G region can be labeled from the cervical cord when the animal matures.

In the diagrams below, the distributions of CS neurons are represented by dots in outlines of fixed brains of normal rats of different ages. (USPHS NS 10531)



195 INFANTILE STIMULATION INDUCES BRAIN LATERALIZATION IN RATS. Victor H. Denenberg, James Garbanati*, Gordon Sherman*, David Yutzey* and Richard Kaplan*. Univ. of Connecticut, Storrs, Conn. 06268

During the first 20 days of life some rat litters were handled daily for 3 min while control litters were not disturbed. When weaned at 21 days half the litters within each group were placed by pairs into standard laboratory cages until Day 70 when all were isolated. The other litters were put into Hebb-type enriched environments until Day 50, then were housed by pairs in laboratory cages until Day 70, and were singly housed thereafter. When approximately 135 days old, rats within each of the 4 experimental treatment groups had (1) a left hemisphere neocortical ablation, (2) a right hemisphere neocortical ablation, (3) a sham operation, or (4) were not distrubed. When 165 days old, all animals were tested for 3 min in the open field for 4 successive days, and their activity recorded.

The activity scores of shams and controls did not differ and their data were pooled. The Handling and the Enriched Environment variables interacted significantly with the Brain Lesion variable. For animals not handled in infancy, regardless of postweaning experience, a cortical ablation in either hemisphere resulted in an increase in activity. In handled animals, ablating the left brain did not affect activity. However, the right brain lesion had complex effects: handled rats reared in laboratory cages after weaning with their right brains ablated were the most active group of all, while handled rats who had had enriched environmental experience after weaning and then had their right brains removed were the least active group.

These findings suggest that, in the rat, handling in infancy results in hemispheric asymmetry of brain organization in adulthood.

196 ORGANIZATION OF THE THALAMIC PROJECTIONS TO THE AUDITORY CORTEX IN GALAGO SENEGALENSIS. D. Fitzpatrick*, D. Raczkowski*, R. J. Ravizza and I. T. Diamond. Dept. Psychol., Duke University, Durham, NC 27706, and Dept. Psychol., Pennsylvania State University, University Park, PA 16802.

In our continuing investigation of the sensory pathways in the prosimian primate, Galago senegalensis, we have examined the connections of the auditory cortex utilizing the retrograde transport of horseradish peroxidase (HRP). Iontophoretic injections of HRP were made in various portions of the auditory cortex and, following a survival time of 24 hrs, the tissue was processed for HRP histochemistry.

Primary auditory cortex (AI), as defined on the basis of cyto- and myelo-architecture, is located on the rostral pole of the temporal lobe, midway between proisocortex and the dorsal extremity of the sylvian fissure. HRP injections in AI resulted in labeled cells in the ventral division of the medial geniculate body (GM_V) which were always arranged in a characteristic laminar pattern. Injections in the dorsal portion of AI resulted in bands of labeled cells along the medial border of GM_V; if HRP was injected more ventrally in AI, the band in GM_V was more laterally located.

Injections of HRP in the different cortical regions surrounding AI led to distinctly different patterns of labeled cells in the thalamus. Injections ventral to AI and dorsal to proisocortex resulted in labeled cells in the caudal extremity of the medial geniculate body. Injections in the lateral bank of the sylvian fissure, medial to AI, produced a prominent focus of labeled cells in the magnocellular division. Injections placed near the dorsal tip of the sylvian fissure led to labeled cells in the suprageniculate nucleus (Sg). Following injections in the cortex intercalated between AI and the target of Sg, labeled cells were observed in the dorsal division of the medial geniculate body and in other portions of the posterior group. Finally, injections in the medial wall of the sylvian fissure produced label primarily in the anterior portion of the posterior group, adjacent to the ventrobasal complex.

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197 INTERHEMISPHERIC CONNECTIONS BETWEEN THE NEOCORTICAL FOREPAW REPRESENTATIONS IN THE VIRGINIA OPOSSUM. Robert E. Foster and Ford F. Ebner. Neuroscience Section, Div. of Bio. and Med., Brown Univ., Providence, RI 02912.

The anatomical distribution of commissurally projecting cells in one hemisphere and their axonal terminations in the other were correlated with the limits of the area showing evoked potentials to electrical stimulation of the forepaw. Within the somatic sensory cortex of the opossum (D. virginiana) the density of commissural cells and their axon terminals varies, but all regions give rise to and receive at least some commissural connections. To chart the distribution of commissurally projecting cells, horseradish peroxidase (HRP) was placed in the transected neocortical commissure. Two days later the forepaw representation on one side was mapped with microelectrodes and small electrolytic lesions were placed along its borders. The animals were perfused and the brains processed to demonstrate the HRP reaction product. The results show that within the central region of the forepaw area only a small number of cells in layers II-III give rise to commissural axons, while along the boundaries of this area such retrogradely filled perikarya are much more numerous and located in all cell layers, with the majority still in layers II-III. The distribution of commissural axon degeneration following large lesions of somatic sensory cortex on one side shows two main patterns. First, within the central region of the forepaw area, dense terminal degeneration is present only in layers I-II and VI, with only sparse degeneration in the other layers. In contrast, the zones adjacent to the central region show dense terminals in layers I through VI. Electron microscopic examination of commissural axons 6-9 days after unilateral decortication showed that their terminals contain round synaptic vesicles and form asymmetrical membrane differentiations in all layers of both regions. Dendritic spines were the most commonly identified postsynaptic element. Thus, even though all parts of opossum somatic sensory cortex give rise to and receive interhemispheric connections, the present results suggest that there are subareas that can be distinguished by the organization of their commissural connections.

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198 THE HUMAN AUDITORY CORTEX: A NEW CYTOARCHITECTONIC MAP. Albert M. Galaburda* and Friedrich Sanides*. (SPON: Gary Van Hoesen) Harvard Medical School, Boston, Ma. 02115.

As a part of a larger study on the microscopic right-left asymmetries in the human brain the primary and association auditory cortices were parcellated in six separate hemispheres using serially sectioned, whole-brain human material stained with the Nissl method. By employing cytoarachitectonic criteria adapted from the Vogt school (F. Sanides, in The Structure and Function of Nervous Tissue, G.H. Bourne, Ed., Academic Press, New York, 1972) the following findings were made: The primary receptive area KA (D.N. Pandya&F.Sanides, Z. Anat. Entwickl.-Gesch. 139:127, 1973) was found mostly on the anterior gyrus of Heschl, and seemed to show varying degrees of focal differentiation, especially in the size of IIIc pyramids; as in the rhesus monkey, belts of association cortex, parakoniocortex, were found surrounding the koniocortex KA. Several divisions of parakoniocortex were noted: a rostral, parvocellular division; an internal division characterized by large IIIc pyramids; a lateral division notable by the presence of rows in layer III; a caudo-dorsal division which is similar to the internal division except for the presence of only foci of large IIIc pyramids and a darker layer V; and finally a temporoparietal division, Tpt, with features of parietal cortex, felt to be a form of integration cortex on architectonic grounds. An area in the parinsular region, proA, making up part of the medial border of KA, was felt to represent a more primitive auditory cortex, A-II.

The morphology of each division, in addition to its location, correspond largely to the auditory areas of v. Economo and Koskinas (Die Cytoarchitektonik der Hirnrinde des erwachsenen Menschen, Springer-Verlag, Berlin, 1925) with one major exception. The caudo-dorsal parakoniocortex was found to extend to the caudal end of the Sylvian fossa and around the back onto the medial aspect of the parietal operculum, there extending rostrally for about one centimeter. To our knowledge this is the first time a cytoarchitectonic map of the auditory cortex has clearly shown the presence of this type of cortex well into the parietal operculum. This data provides for the first time a cytoarchitectonic substrate for the neurophysiologic findings of auditory evoked potentials in the parietal operculum (T.J. Imig et al., J. Comp. Neurol. 171:111, 1977).

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199 EFFECTS OF DIPHENYLHYDANTOIN ON EVOKED POTENTIALS AND SINGLE NEURON ACTIVITY IN THE ANTERIOR CEREBRAL CORTEX OF DOMESTIC CATS. F.A. Harris and A.L. Towe. Dept. Physiol. & Biophys. (SJ-40), Univ. of Wash. Sch. Med., Seattle, WA 98195.

Surface-recorded gross potentials and extracellularly recorded single neuron activity evoked by skin stimulation were studied in pericruciate and precoronal cortex of chloralose-anesthetized, immobilized cats before and after topical application of diphenylhydantoin to each recording site. A minute amount applied in crystalline form produced an immediate and profound reduction in evoked potential amplitude, followed by recovery along a time course suggestive of spreading cortical depression. Occasionally, after an extremely small amount of the drug was applied, the gross potential evoked by contralateral forepaw (CF) stimulation increased, "diphenylhydantoin spikes"—gross potentials analogous to strychnine and bicuculline "spikes"—appeared after off-focus (IF, CH and IH) stimulation, and the evoked discharge of single neurons increased to CF stimulation (sa neurons) or to all inputs (m neurons). In precoronal cortex, the effects of the drug applied in saline solution at 1 mg/ml, buffered to pH 7.4, included a brief initial reduction in gross potential amplitude, followed by recovery (about 2 min) and then an increase lasting from 15 min to 1.5 hr. In pericruciate cortex, the same effects occurred in response to CF stimulation. In addition, "diphenylhydantoin spikes" appeared, appended to the normal gross potentials evoked by off-focus (IF, CH and IH) stimulation. The evoked discharge of single neurons increased, generally in parallel with the enhancement seen in the gross potential evoked by CF stimulation. There were a few paradoxical instances in which neuron response increased while the simultaneously recorded gross potential decreased. One precruciate neuron changed from an sc type (response only to CF and CH) before drug application to an m type (response to all paws) after drug application. It remains to be explained why an agent which is used chronically as an anticonvulsant drug, should produce "convulsant" activity, like that produced by strychnine and bicuculline, when applied acutely to the surface of the cortex. The increased excitability of single neurons after topical application of the drug may bear a relation to the increased tendency to seizures associated with abrupt withdrawal of the drug in persons under chronic therapeutic treatment (i.e., there may be a critical local concentration at which this drug has an excitant effect). (Supported by NS00396 and NS05136)

200 MEASUREMENT OF CELLULAR AND EXTRACELLULAR PARAMETERS OF CEREBRAL CORTEX BY ANALYSIS OF CORTICAL ELECTRICAL IMPEDANCE. James W. Havstad* (SPON: Robert P. Scobey). Department of Pharmacology, School of Medicine, University of California, Davis, CA 95616.

Electrical impedance of cerebral cortex has often been considered to be determined by extracellular current flow, thereby providing little information about cellular elements. Ranck (Exp. Neurol. 9:1-16, 1964) has obtained a different conclusion using an analysis based on randomly oriented, branching cellular elements. This problem has been reexamined by measurement of magnitude and phase angle of impedance of superficial cortex in the rabbit. From anatomical and passive electrical characteristics of the superficial cortex, a mathematical model of cortical impedance was developed, which resembles that of Ranck in assuming random dendritic structures. Comparison of experimental data with prediction from the model supports the conclusion that impedance is a measure of both cellular and extracellular parameters.

Impedance magnitude and phase angle are measured substantially continuously and simultaneously at one-half decade frequency intervals from 5Hz to 15.8kHz, using a four-pole electrode array and subthreshold currents. The electrodes rest on the exposed pial surface, and measure impedance to a depth of about 0.25 mm. Impedance magnitudes are about five times the resistivity of isotonic saline and decrease with increasing frequency. Phase angles are negative and show peaks of about 2.4 degrees around 50Hz and 3 degrees at 15.8 kHz. Good agreement with the model is found when a finely lamellar structure for glia is assumed. The low frequency phase angle is due to current flow in dendrites oriented substantially parallel to the measuring field, and is a measure of dendritic membrane time constant. The high frequency phase angle peak is due to similarly oriented glia, but is independent of glial membrane resistance.

Analysis of impedance changes during spreading depression shows the expected changes in dendritic membrane permeability and extracellular volume, and allow monitoring of their individual time courses. An additional unexplained change in dendritic membrane is required during recovery from spreading depression to account for prominent features in the experimental data.
(Supported by NIH grant MH17471).

201 LIGHT AND ELECTRONMICROSCOPIC ANALYSIS OF INTRINSIC NEURONS IN CATS SUPRASYLVIAN GYRUS; DEGENERATION PATTERNS AFTER CORONAL LESION. V. Hayes*, B. Weissman*, I. Kaiserman-Abramof, J. Ferguson. Division of Neurology and Department of Anatomy, Case Western Reserve University School of Medicine, and Veterans Administration Hospital, Cleveland, Ohio.

To determine the origin, extent, and termination of neurons intrinsic to cat suprasylvian gyrus (SS), light and electronmicroscopic (EM) sections of degeneration patterns were studied after a transverse (coronal) subpial cortical lesion was made by a fine wire leukotome. Fink-Heimer sections at 3 days showed maximal terminal degeneration in layers I and II up to 8 mm anterior and posterior from the lesion. Fiber degeneration, maximum at 7 days, extended 10 mm anterior and posterior from the lesion, was mostly horizontal and mainly restricted to layers III-VI.

For the EM study, counts of layer II neuron soma and neuropil terminal degeneration in a 29/39μ area around each cell were made 1 and 2 mm from the lesion.

Survival time (days)	Areas analyzed	Terminal/ area	% Degeneration	Cells analyzed	Terminals/ Soma section	% Degeneration
Control	11	88	0	13	7	0
2	8	84	1	10	8	2
4	10	81	1	10	4	0
21	8	98	2	8	4	2

The degeneration shown in the table above represents dark degeneration and was 2% or less in all areas for cell soma and neuropil. A significant decrease in axon terminals/cell soma/area (ATCA) occurred between the second and fourth day and was still present at 21 days. This abrupt and lasting decrease in ATCA numbers could represent terminal degeneration with removal over the period studied. In addition to the decrease in ATCA, the observation that glial processes occupied increased surface areas of neuron somata may indicate glial removal of degenerating terminals and occupation of the vacated terminal sites.

These EM data for layer II SS in the cat argue that the major effect of a single coronal lesion is the removal of terminals from neuron cell bodies. The Fink-Heimer study suggests that the soma terminal loss could extend for over 16 mm in SS.

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202 ROLE OF VISUAL CORTEX IN ANTICIPATORY ORIENTATION TOWARD MOVING TARGETS BY THE GERBIL. David Ingle, Brandeis University, Waltham, MA 02154

Throughout the vertebrate phylogeny, the optic tectum appears to mediate rapid orientation of eyes or head toward objects of interest to the animal. We have developed a procedure for cine analysis of visually-elicited orientation by the Mongolian Gerbil (Meriones unguiculatus) by which subtleties of visuomotor behavior can be discovered and analyzed quantitatively. Repeated analysis of six normal gerbils, trained to wait for the sudden appearance of discs baited with seeds, reveals that the initial head turn typically undershoots a stationary target. However, when stimuli emerge from behind frontal barriers and move toward the periphery, the gerbil's first head turn is typically an overshoot of the target position seen at the initiation of the turn. That is to say, the gerbil's turn leads a moving target and sets him on a collision course.

Two gerbils with large lesions of visual cortex made normal initial head turns toward stationary objects throughout their visual fields. However, both animals consistently failed to show the "anticipatory" overshoot of head movement (and subsequent pursuit) associated with temporalwards moving objects. Further studies are in progress, using unilateral lesions more nearly limited to striate cortex. These data confirm the qualitative observation of Schneider (Science, 163:895-902, 1969) that hamsters can orient accurately following large lesions of visual cortex. They add the new information that "tracking" of a rapidly moving target depends upon integrity of cortex as well as of the optic tectum. Our observations are in agreement with those of Humphrey (Perception, 3:241, 1974), who noted that a long-term destriate monkey was capable of accurate eye saccades but showed no smooth pursuit abilities. It seems likely that a cortically triggered mechanism can "override" the simpler orienting behavior mediated by the optic tectum during pursuit of moving targets.

203 RECIPROCAL CONNECTIONS OF THE THALAMUS AND THE INFERIOR PARIETAL LOBULE. David L. Kasdon* and Stanley Jacobson. Departments of Neurosurgery and Anatomy, Tufts Univ. Sch. Med., Boston, Mass., 02111.

The inferior parietal lobule (IPL) of the rhesus monkey represents association cortex with multimodal sensory input subserving language, visual and related higher cortical function. We have studied the IPL with eight cortical injections of HRP demonstrating diverse thalamic input to this region (Anat. Record 187:769). A major projection to the superior portion of the IPL was from the anterior nuclei and Paracentralis of the intralaminar group. Ventralis Lateralis and oral Pulvinar projected primarily to the anterior-inferior portion of the IPL, whereas Lateral Posterior projected most strongly to the anterior and superior portion of the IPL and to area 19. The projections of the inferior Pulvinar were heaviest to area 19, but there was some overlap in the mid-superior portion of the IPL with the medial and lateral Pulvinar. The major projection from the posterior thalamic complex was to the mid-IPL. In addition to this retrograde HRP study, tritiated amino acids were also injected in the same eight parts of the IPL and the brains processed for autoradiography so that the reciprocal anterograde connections of the IPL to the thalamus could be compared to the HRP material. The heaviest projections from the thalamus to the IPL were reciprocated by strong projections from the IPL to the thalamus.

204 VERBAL CROSS-CLUEING FOLLOWING CEREBRAL COMMISSUROTOMY. Santosh Kumar, Glenda M. Bogen* and Joseph E. Bogen. Ross-Loos Medical Center, Los Angeles, CA 90026.

Following complete cerebral commissurotomy for medically intractable epilepsy (Bogen & Vogel, 1962), behavior seems normal in ordinary social situations and in routine neurological examination (Bogen & Vogel, 1975). But specific deficits can be demonstrated using special tests (Sperry, Gazzaniga & Bogen, 1969); these include an inability to retrieve correctly with one hand a test object originally presented to the opposite hand. The disability for cross-retrieval in spite of excellent same-hand retrieval demonstrates both the capacity of each cerebral hemisphere to function independently, and a lack of interhemispheric transfer of discriminative information. Cross-retrieval from right hand to left is improved if naming out loud is permitted during palpation with the right hand; this has been attributed to the right hemisphere's ability to hear and comprehend spoken words. A recent, quantitative, systematic study affords comparisons between subjects for this verbal cross-clueing strategy and confirms its presence for a decade or more after operation.

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205 EFFECT OF DIMETHYL SULFOXIDE ON CEREBRAL INFARCTION IN THE MONGOLIAN GERBIL. Jerry W. Lawson and Charles P. McGraw, Dept. Neu. Bowman Gray Sch. Med. Wake Forest Univ., Winston-Salem, NC 27103

Dimethyl sulfoxide (DMSO) has several characteristics that should enable it to improve morbidity and mortality in acute cerebral ischemia: 1) as a strong diuretic it should reduce blood volume, intracranial pressure, and tissue edema, and 2) as a vasodilator it should improve cortical blood flow. DMSO has been reported to be beneficial in the treatment of central nervous system trauma, possibly due to its reported anti-inflammatory, antiedemic, anticoagulate, diuretic, hypothermic, vasodilatory, and respiratory stimulatory effects as well as to its ability to correct membrane instability and penetrate the blood-brain barrier. de la Torre and Surgeon reported that in rhesus monkeys subjected to experimental cerebral infarction, DMSO-treated animals had less brain damage and greater protection from the severe neurological deficits seen in control animals.

Two hundred and thirty-four young adult Mongolian gerbils of both sexes weighing 40 to 60 grams were divided into three groups: Group 1 (180 animals operated on, one-half DMSO-treated and one-half saline-treated); Group 2 (42 animals sham-operated on, one-half DMSO-treated and one-half saline treated); and Group 3 (12 animals not operated on, DMSO-treated). The animals operated on were anesthetized with either ether or ketamine. A ventral midline cervical incision was made from the mental protuberance of the mandible to the manubrium. The left common carotid artery was located by blunt dissection, care being taken not to injure the jugular vein or the adjacent nerves. In Group I animals, the artery was doubly ligated with 5-0 monofilament nylon suture and transected between the ligatures. Then, in all animals, the incision was closed with 9-mm stainless steel autoclips. Each animal was then given a 1-ml intraperitoneal injection of 1% trypan blue solution. In one-half of the animals in the two operated groups and in all 12 control animals, 0.5 ml of DMSO solution was injected intraperitoneally one hour post-ligation. Then, every eight hours for 72 hours, either 5 or 2.5 gm/kg of DMSO solution was injected intraperitoneally by a double-blind protocol. The other half of the animals in the operated groups received saline injections on the same treatment schedule. Clinical signs of infarction were recorded. The accumulative probability of dying was calculated.

DMSO had no significant effect on the number of infarctions, mortality, or the extent of clinical morbidity. However, it did significantly decrease ($p < 0.05$) the amount of trypan blue staining in animals with infarction. A 10 percent incidence of drug toxicity in animals receiving the higher dose was observed.

206 PREFRONTAL CORTICOFUGAL PROJECTIONS IN MACAQUE MONKEYS. George R. Leichnetz, Juan Astruc and John T. Povlishock*. Department of Anatomy, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia 23298.

Anterograde degeneration studies using the Nauta and Fink-Heimer silver techniques following unilateral partial ablations of the medial prefrontal cortex (Area 9) in rhesus and cynomolgus monkeys have led us to the conclusion that this region has corticocortical projections to Area 6, superior temporal cortex (Area 22), superior and inferior parietal cortices (Areas 5 and 7), prestriate area (Area 19), entorhinal area (Area 28a) and, via the cingulum bundle primarily, to the hippocampus (primarily caudal CA_3). This region gives rise to subcortical projections to the corpus striatum, including the head of the caudate nucleus (but also the vertical portion of the tail), although the putamen is notably lacking in degeneration. The dorsal globus pallidus is traversed by large numbers of degenerating fibers of passage, some of which is suggestive of preterminal and terminal debris. The basal and lateral amygdaloid nuclei likewise receive medial prefrontal input. In the thalamus some preterminal degeneration was present in the anterior nuclear complex, caudal MDpc, LD, LP and to a much lesser extent in the pulvinar. The central lateral and parafascicular nuclei contained moderate to heavy amounts of degeneration. Brain stem projections were observed to the pretectum and tectum, and the paramedian mesencephalic reticular formation (midbrain limbic area). Recent electron-microscopic studies have provided evidence thus far confirming direct prefronto-hippocampal and prefronto-amygdaloid projections. EM verification of other prefrontal target areas is under way. Likewise, current autoradiographic investigations have substantiated in large measure the findings of the silver studies. Supportive EM and autoradiographic evidence will be presented. In addition, comparisons will be drawn to ongoing investigations of the efferent connections of the dorsal and ventral convexity, and the possible diversity of roles of the various prefrontal subregions will be discussed. This study has been supported in part by USPHS NB 08418 and A. D. Williams Research Grants.

207 CLAUSTRUM TO FRONTAL CORTEX CONNECTIONS IN THE CAT, STUDIED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE (HRP). Alfonso Llamas*, Carlos Avendano* and Fernando Reinoso-Suarez* (SPON: D.A. Pasquier). Departamento de Morfologia, Facultad de Medicina, Universidad Autonoma de Madrid, Spain.

The distribution of HRP labeled neurons in the claustrum has been investigated as part of a broader study of the afferent connections of the frontal cortex in the cat.

Nineteen adult cats were selected for this study. In 11 of these animals 0.3 to 2.0 microliters of 30% aqueous solution of HRP (Sigma VI) were injected in various parts of the prefrontal, premotor and motor areas; the remaining eight cats received injections in other regions of the cerebral cortex in order to get a general view of the claustrum-cortical connections. After the survival time of 36 to 50 hr. animals were perfused and processed according to the method of Llamas and Martinez-Moreno (An. Anat. 23: 431, 1974)

The results can be summarized as follows: 1) all the injections gave rise to labeled neurons in the dorsal (insular) claustrum; 2) the ventral, or prepiriform claustrum, was always free of labeled cells; 3) the more rostral and dorsal injections in the frontal cortex (dorsal part of gyrus proreus and rostral part of gyrus sigmoideus anterior) labeled neurons in the dorsal and rostralmost part of the claustrum; 4) the more ventral and rostral injections in the medial surface of the hemisphere gave rise to labeled neurons in the rostroventral extreme of the claustrum; 5) more caudal injections in the motor cortex (including both banks of the sulcus cruciatus) labeled cells in progressively more caudal sections of the claustrum, always in its dorsal one half or one third.

As a rule, more caudal injections in the cortex produce labeling in more caudal locations in the claustrum; and dorsal injections, both in the convexity and the medial surface, produce labeling of the dorsal part of the claustrum. A ventral-to-ventral topometry was also found in the convexity and in the medial surface of the hemisphere.

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208 TOPOGRAPHICAL ORGANIZATION OF THE THALAMIC PROJECTION FROM THE MOTOR CORTEX OF THE RAT. L. C. Massopust, P. A. Young, and C. J. Seliga*. Dept. Anat., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Considerable attention has been given to the convergence of pallidal, cerebellar, and cortical projections in the ventral tier thalamic nuclei of gyrencephalic animals. Similar studies in lissencephalic animals such as the albino rat are meager. Utilizing selective silver impregnation and autoradiographic techniques, electrolytic lesions or injections of tritiated amino acids were made at 4 predetermined sites in the motor cortex as localized in the physiological maps of Hall and Lindholm (1967). From the anterior two sites, degenerating or labelled fibers entered the white matter, coursed through the caudate-putamen complex and internal capsule, and then penetrated the dorsolateral aspect of the anterior part of the thalamus. Terminal areas were seen in the reticular, ventral anterior, and intralaminar (IL) nuclei. More posteriorly, terminal areas were concentrated in the IL, in the dorsomedial part of the ventral dorsal nucleus (VD), in the dorsolateral part of the ventral medial nucleus (VM), and along the extreme lateral border of the medial dorsal nucleus (MD). From the posterior intermediate site, corticothalamic terminal areas were observed in the reticular, IL, and along the lateral border of the ventral lateral nucleus (VL). Further posteriorly, terminations formed a sharply localized "comma-shaped" area near the ventromedial border of the VL. Lesions or injections in the most posterior part of the motor cortex exhibited projections and terminations similar to the posterior intermediate except that the comma-shaped terminal area was located more laterally and dorsally in the VL. Because of these connections with the VL it is postulated that the posteromedial part of the cortical area described by Hall and Lindholm is the primary motor area (area 4). Since the anterior part of this cortical area projects largely to the IL, VD, and VM, it may correspond to the premotor cortex (area 6). Other investigations in our laboratory show that somatosensory cortical, pallidal, and cerebellar projections terminate topographically in the ventral tier nuclei and intralaminar areas, thus showing an organization similar to that in higher mammals.

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209 STUDIES ON CAUDATE NUCLEUS INHIBITION OF SEIZURE ACTIVITY IN THE LIMBIC SYSTEM AND CEREBRAL CORTEX. R. J. Morgan, G. T. Schneider* and C. C. Turbes. Colorado State University, Ft. Collins, CO 80521 and Creighton School of Medicine, Omaha, NE 68178 U.S.A.

Multiunit and slow wave activity were recorded from extracellular microelectrodes in the dorsal hippocampus and amygdala in twenty-eight cats. The electroencephalogram was recorded from the right and left frontal, parietal temporal and occipital cerebral cortex. Three channels were recorded on an F.M. tape recorder for further data analysis. The data was processed with a Varian V-72 minicomputer. Channels were sampled alternately at 2.5 ms and 120 data segments for each channel of 2.56 seconds duration each. These data were transformed using a Fast Fourier Transform algorithm to give a power spectral estimate. Stimulating electrodes were located in the left septal nucleus, stimulation produced after discharge at cerebral cortex and the limbic system. Generalized seizures were induced with pentylenetetrazol. Focal application of penicillin to the cerebral cortex was used to produce a focal seizure. Stimulation of the caudate resulted in inhibition of septal afterdischarge and generalized seizure activity in the cerebral cortex and the limbic system. Caudate stimulation caused desynchronization of synchronized seizure activity induced by local application of penicillin to the cerebral cortex.

210 VARIATIONS IN THE FISSURAL PATTERN OF THE CEREBRAL NEOCORTEX OF THE SPIDER MONKEY (ATELES). Benjamin H. Pubols Jr. and Lillian M. Pubols. Dept. of Anatomy, College of Medicine, Pennsylvania State University, Hershey, PA, and Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA.

The existence of variations in the fissural pattern of the neocortex of primate brain specimens has been recognized for well over 100 years (Gratiolet, 1854; Huxley, 1861). Since then, a number of observers have described the external features of the neocortex of Ateles, but little attention has been paid specifically to the question of interhemispheric variability, either between or within specimens of the same genus.

The present report is an attempt to characterize and categorize 40 hemispheres of 22 specimens of Ateles acquired during the course of a series of previously published investigations of the somatic sensory system of this species. The fissural patterns of the dorsolateral neocortical surface have been classified according to certain salient features illustrated below.

Fig. 1 illustrates a synthetic "typical" hemisphere (left dorsolateral view), in which (1) the caudal spur of the inferior precentral sulcus (pci) comes to within 5 mm of, but is discontinuous with the base of the central sulcus (c) (68% of cases), (2) the superior (pcs) and middle (pcm) precentral sulci are discontinuous (83% of cases), (3) the transparietal (tps) and superior postcentral (pts) sulci are discontinuous (65% of cases), and (4) there is no true superior frontal sulcus (fs) (40% of cases). Fig. 2, also synthetic, illustrates a few of the variations on this pattern: (1) pci and c are continuous, (2) pcs and pcm are united to form a single sulcus, (3) a transversely oriented fs is present, and (4) tps extends anteriorly across the postcentral gyrus to separate regions of representation of arm and leg, as defined by electrophysiological studies (Pubols and Pubols, 1971). Three other hemispheres also showed such somatotopically related transverse fissures, in either precentral motor, or postcentral sensory cortex. In still other hemispheres, tps and pts joined to form a triradiate sulcus. Interhemispheric variability was as pronounced within as between specimens.

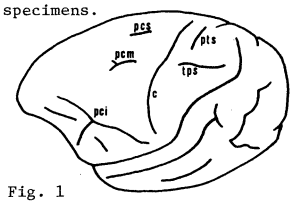

Fig. 1

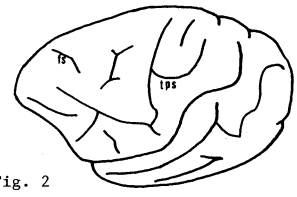

Fig. 2

211 SOME OBSERVATIONS ON LAMINAR ORIGINS AND TERMINATIONS OF OCCIPITO TEMPORAL CORTICAL CONNECTIONS IN RHESUS MONKEY. K.S. Rockland* and D.N. Pandya. Dept. Anat., Boston Univ. Sch. Med., Boston, Ma. Neurological Unit, Beth Israel Hosp.; Bedford V.A. Hosp.

Laminar origins and terminations of cortico-cortical connections were studied using anterograde (tritiated amino acids) and retrograde (horseradish peroxidase) transport methods. In general four differential patterns of laminar connectivity could be identified. 1) In sequential forward connections, axon terminals were observed mainly around layer IV. The cells of origin of these sequential projections were localized primarily in the supragranular layers, with some degree of variability in different architectonic areas. Thus, pyramidal cells of L. IIIa,b of area 17 (a few in L. IIIc) projected around L. IV of area 18; neurons of L. IIIc,b of area 18 (a few in L. IIIa and infragranular layers) projected around L. IV of area 19; and, conforming to the supragranular trend, cells in L. IIIb,c of area 19 (some in L. IIIa and infragranular) sent terminals rostrally to L. IV of inferotemporal cortex (area 21). 2) Each of these areas (i.e. areas 18, 19, 21) also projected caudally to the immediately adjoining region. Terminations in this instance, however, were confined primarily to the plexiform layer. Unlike the supragranular preference of rostrally directed projections, the cells of origin of these caudally directed projections were generally found in both supra- and infragranular layers, with some interregional variability. Thus, L. I of area 19 received projections from both areas 21 and 20 (area TF of Bonin and Bailey). The cells of origin of the first-layer projection from area 21 were found in both supra- and infragranular layers (in Ls.IIIa,b and Vb,VI), while neurons in area TF occurred almost exclusively in infragranular layers. The L. I projection from area 19 to area 18 originated from pyramidal cells in both supra- and infragranular layers (Ls.IIIb,a and V,VI). 3) Laminar origins and terminations of intra-regional projections varied widely. In the anterograde direction, local area 17 projections ended in Ls. I and V, with some terminals in L. IIIc, while in areas 18 and 19, these were limited to L. I. Distant intra-19 projections (from preoccipital gyrus to lunate, superior temporal, intraparietal, occipito-temporal sulci) predominantly terminated in Ls. I to IV. 4) Neurons giving rise to callosal connections occurred in L.III: large pyramids in L. IIIc of juxtastriate cortex, and medium to large neurons in L. IIIc,b of area 21. Thus, sublaminar specialization within layer III may be occurring, since layer III neurons are also involved in both rostrally (L. IIIc,b) and caudally (L. IIIa,b) directed cortico-cortical connections.

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212 MEDIAL PREFRONTAL CORTEX AND BEHAVIOR OF DOGS. Carl E. Rosenkilde and Waclawa Lawicka*. Dept. of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland.

A group of mongrel dogs was trained on a go-left/go-right time discrimination task involving 10 and 30 sec of bodily restraint. Another group learned a spatial delayed alternation task in which the animals were restrained during the delays. The effects of two selective prefrontal ablations were evaluated in retention of these tasks. A dorsal lesion comprised the proreal gyrus, while a medial lesion involved the precruciate area but spared the anteromedial field.

Normal dogs showed systematic bodily orientation during the periods of restraint in both situations. No behavioral changes resulted from dorsal prefrontal lesions, but medial damage disturbed positional mediation and instrumental choice in time discrimination as well as in delayed alternation. These deficits are unrelated to the delayed response impairment which previously has been reported after dorsal but not medial lesions. Interference with spatial responding based on kinesthetic cues may account for the present effects of medial prefrontal damage.

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213 DESCENDING PROJECTIONS TO THE REGION OF THE GIGANTOCELLULAR TEGMENTAL FIELD OF THE MEDULLA OBLONGATA. Jerome Siegel, Patricia M. Saxton* and Timothy Hageman*. Inst. for Neuroscience and Behavior, Univ. of Delaware, Newark, DE 19711.

The gigantocellular tegmental field (FTG) of the medulla exerts inhibitory influences in both ascending and descending directions (Brodal, Neurological Anatomy, 1969; Mancia et al., Brain Res. 25, 1971, 638). There is evidence that inhibition produced by stimulation to some forebrain structures is mediated by this bulbar inhibitory area (Sauerland et al., Brain Res. 6, 1967, 164). This study was undertaken to identify those regions in the rostral parts of the brain which have direct projections to the bulbar reticular formation. In rats and cats HRP was injected into the bulbar brain stem in the area of the FTG. In all animals it was found that cortical cells which project to the bulbar brain stem are limited to the frontal region. In the cat these areas are precruciate-anterior sigmoid, coronal and proreus gyri (areas 6 and 8). In the rat labeled cells were found in the frontal lateral dorsal cortex (area 2). In both species the HRP positive cells were located bilaterally in clusters of 3 - 15 cells and were always limited to the deep layers of the cortex. It is striking that the only cells in the cerebral cortex in the cat which project to the bulbar area are totally included within cortical areas that have been demonstrated to exert inhibitory influences upon behavior (Wilcott and Sabol, Neuropsychol. 15, 1977, 155).

Cells in the central nucleus of the ipsilateral amygdala were labeled in animals in which the HRP injection site included the central-lateral bulbar brain stem. This confirms the report by Hopkins (Neurosci. Lett. 1, 1975, 263). The amygdaloid projection was much more evident in rats than in cats. Only one cat (with the largest HRP injection: 1 µl) showed some labeled cells in the amygdala and the ventral part of the mesodiencephalic junction, while all rats had the amygdaloid label and also exhibited two discrete clusters of labeled cells in the hypothalamus. One was a periventricular dorsal group with a medial-lateral distribution of cells. More caudally there was a ventral tier of cells above the mammillary complex.

At the level of the midbrain all animals showed a prominent label in the deep layers of the contralateral superior colliculus. In addition, cells in the oculomotor complex and surrounding central gray and the reticular formation at all brain stem levels were clearly labeled. The most caudal cells, which were labeled by axoplasmic transport rather than by direct filling, were located in the contralateral vestibular complex.

(Supported by NSF Grant BN 76-01652.)

214 VASCULAR RESPONSE AND LEVEL OF OXIDATION OF CYTOCHROME a,a_3 IN ASTROCYTOMAS IN SITU. A. Sylvia, M. Rosenthal, D. Bigner* and G. Somjen, Dept. of Physiology and Pharmacology and Microbiology, Duke University Medical Center. Durham, N.C. 27710

Tumors were induced by inoculating either cerebral cortex or subcutaneous tissue of rats with a suspension of the cultured pure astrocytoma cell line RG 2 developed by Dr. W. Wechsler. Four to six days later, the rats were anesthetized with pentobarbital, and the tumor exposed by opening the skull but not the dura. Dual wavelength reflectance spectrophotometry was used to record changes in the redox ratio of cytochrome a,a_3 (605-590 nm), the oxygenation level of hemoglobin (577-585 nm) and the relative volume of blood in an area approximately 3 mm in diameter and 1 mm in depth in intact cortical tissue in situ. The rats were immobilized with tubocurare and, in the control state, ventilated with 30% O_2 and 70% N_2. Addition of CO_2 to the inspired gas mixture caused vasodilatation in the glioma in the brain, but not when the glioma was located in subcutaneous tissue. This indicates that vessels invading the tumor retain the regulatory responses typical of the body region, and do not acquire those typical of the tissue of origin of the neoplastic cells. In addition, rhythmic vasomotion was a regular finding in the gliomas in skin but not in brain. The redox ratio of cytochrome a,a_3 in the unstimulated, unperturbed control state was compared to that during breathing 100% O_2 and 100% N_2. Unlike normal cerebral tissue in which cytochrome a,a_3 is at least 30% reduced in the unstimulated state, in astrocytoma tissue reduction levels varied from 0 to 15% regardless of whether the glioma was located in skin or brain. The 30% or greater reduction levels were found not only in the cortex of normal control rats, but also in the untreated hemisphere of inoculated rats, and also in normal cortex adjacent to the tumor on the inoculated side. In having nearly fully oxidized cytochrome a,a_3, the mitochondria of astrocytoma cells resemble isolated mitochondria, and differ from those of cells in non-neoplastic brain tissue in situ. It may be that the neoplastic cells lack a regulatory or control function typical of normal brain cells. Alternatively, the values measured in normal brain may be intermediate between neuron and glial population, and the astrocytoma cells may reflect the normal state of glia.

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215 PULVINAR AND OTHER CAUDAL THALAMIC AFFERENTS TO SUPERIOR TEMPORAL GYRUS IN THE RHESUS MONKEY. John Q. Trojanowski, Dept. of Anatomy, Tufts University School of Medicine, Boston, Ma. 02111

In five juvenile rhesus monkeys, using the horseradish peroxidase (HRP) technique, pulvinar and other caudal thalamic afferents to the superior temporal gyrus (STG) were examined. As in a similar series of experiments in the squirrel monkey (Brain Res., 85:346-353) the HRP was combined with tritiated amino acids for later study of the efferents of STG. In the five animals, unilateral injections of the tracers were made such that the injection sites in total involved the entire length of STG. The material was processed in the usual manner to reveal HRP labeled neurons and the location of these neurons was charted with an x-y pantograph. The HRP results in the Rhesus monkey accord well with those previously described in the squirrel monkey (Brain Res., 85:347-353). As far as the pulvinar is concerned, the anterior third of STG primarily receives projections from the caudal part of the ventromedial portions of medial pulvinar nucleus while the posterior third of STG receives projections both from the medial and lateral pulvinar nuclei. The middle third of STG, into which secondary auditory cortex extends, receives the bulk of its input from the medial geniculate nucleus rather than from the pulvinar. As noted in the squirrel monkey study, several other caudal thalamic nuclei were found to project to STG as well, i.e., the medial dorsal and suprageniculate nuclei and nucleus limitans. Thus in the Rhesus monkey as in the squirrel monkey, it is the pulvinar nucleus in caudal thalamus which is the primary source of input to STG exclusive of the primary and secondary auditory areas. The relationship of pulvinar to STG is topographical and paralleled by less well organized projections from other caudal thalamic nuclei.

216 ORGANIZATION OF THE PRIMATE AND HUMAN CEREBRAL CORTEX STUDIED WITH THE DIRECT CORTICAL RESPONSE. John Turner*, Christer Lindquist*, and Robert G. Grossman. Depts. Neurosurg., Inst. of Neurol. Sci., Glasgow, and Univ. Texas Medical Branch, Galveston, TX. 77550.

The organization of cortical cytoarchitectonic areas was studied with the direct cortical response (DCR) in 27 baboons and subsequently in 30 adult patients who underwent electrocortical mapping to localize functional areas of the cortex as a guide to neurosurgical procedures. The DCR of the primate and human cortex is considerably more complex than that of lower mammals. The fully developed DCR is a mosaic consisting of a series of potentials that have different thresholds, latencies and spatial distributions. The amplitudes and latencies of the potentials are a function of the gyrus studied, stimulus-recording distance, and stimulus intensity. When studied at S-R distances of 6-9 mm, the fully developed DCR consists of a positive wave (P_1) occupying the initial 4 msec after the stimulus, a biphasic negative wave (N_{1a}, N_{1b}) with peaks at 8 and 16 msec in the baboon and 12 and 25 msec in the human, a large positive potential (P_2) with a peak at 40-60 msec, and a late slow negative wave with a peak at 125-150 msec. Differences in the organization of eulaminate, agranular and koniocortices are revealed by the DCR. The morphology of the response is simplest in the frontal eulaminate cortex, where P_1 is small or absent, N_1 is prominent, the distinction between N_{1a} and b is not marked, and P_2 is not prominent. The agranular motor cortex can be identified by a series of 1.5 msec duration notched waves riding on P_1 (a,b,c). The somatosensory koniocortex can be identified by a more complex series of wavelets inscribed on the P_1 wave. The visual cortex also has a complex P_1 morphology that is not present in the surrounding parietal cortex. On the basis of differential sensitivity to ischemia ($N_1 > P_2 > P_1$) and strength-duration, conditioning-testing, and field potential analysis we tentatively assign the origin of N_1 a and b to EPSPs in elements in the upper cortex; P_2 to IPSPs in elements in the upper cortex, and P_1 to EPSPs in deeper elements. The early portion of P_2 contributes to the total amplitude of the early P_1 positivity. P_1, a,b,c, may be presynaptic fiber responses. The DCR can be used clinically for identifying cortical gyri, and DCR morphology and interaction with spontaneous potentials provides a tool for studying cortical organization.

217 SELECTIVE INHIBITION OF SOME WIDE-FIELD SENSORIMOTOR CORTEX NEURONS BY HIGH-INTENSITY SKIN STIMULI. C.F. Tyner and M.G. Miller*. Dept. Neurosciences, Walter Reed Army Inst. Res., Wash. DC 20012.

We studied single neurons extracellularly in forepaw postcruciate (4*) cortex in chloralose-anesthetized cats and held arterial blood gas levels normal. We found cells by serially shocking the four limbs' central footpads (needle electrodes, 30 milliamp (ma)(13.5 v), 0.1 msec, 1/sec) during microelectrode advance. Prior study of this tissue (Towe, et.al., Exp. Neurol., 50, 734, 1976) has emphasized two neuron populations: sa cells (superficial, contralateral forelimb (CF) receptive fields, mostly touch sensitive, few pyramidal tract (PT) axons); and m cells (deeper, whole-body receptive fields, mostly hair sensitive, providing most PT axons).

We placed most neurons from our sample (n=170 to date) into the sa/m classification, but found about 13% of cells in this tissue unresponsive to 30 ma CF shock although responsive to other stimuli. Most of these i neurons, about 10% of the total sample, responded to stimulation of ipsilateral and hindlimb footpads, contralateral forelimb shoulder, and both splanchnic nerves: they thus closely resemble m neurons. Further study showed nearly all wide-field i cells responsive to low-intensity CF shock with thresholds similar to those of m cells (1.0-4.5 ma); but unlike m cells they ceased responding to CF shock at intensities above 8-15 ma.

Single-blind shock stimulation of the experimenters' skin (intradermal needles, 0.1 msec, 1/sec) showed sensory thresholds of about 0.9 ma (0.4 v) with a transition from moderately irritating to clearly painful sensations in the 6-14 ma (2.7-6.3 v) range; from comparing these results with those of Collins, et.al. (Arch. Neurol., 3, 381, 1960), we infer that A-delta but not C fibers are probably recruited over the 6-14 ma span.

Most m neurons in this tissue show unexpected behavior following CF stimulation, perhaps due to modulation by nearby sa cells (Towe, et.al., 1976). We envision the wide-field i cells as a significant subset of the m population -- perhaps as large as 20% -- which receives potent inhibition following intense stimulation of the topographic skin focus, and believe their behavior may represent the modulation of corticofugal activity by painful stimuli.

218 BRAINSTEM PROJECTIONS TO CEREBRAL CORTEX IN WATER SNAKES. Philip S. Ulinski. Dept. Anat., Univ. Chicago, Chicago, IL 60637.

It is now well established that several brainstem structures project to the cerebral cortex in mammals. However, comparable projections in reptiles have received less attention. The possible existence of brainstem projections in banded water snakes (Natrix sipedon) was investigated following unilateral transections of the midbrain designed to interrupt all ascending fiber systems. Animals survived for seven to 28 days and their brains were then prepared by the Fink-Heimer procedure. The experiments demonstrate a large contingent of axons which reach the forebrain via the medial forebrain bundle. A minority of the fibers decussate over the anterior commissure. They run along the medial surface of the septum on both sides of the brain and gain access to the cortex through the alveus. They pass through medial cortex (Ulinski, 1974, J. Comp. Neur., 158: 243) and begin to slant upwards as they approach the medial edge of dorsomedial cortex (DM). They pass through layer 2 of DM and turn to run in the inner two thirds of layer 1 of DM. They, therefore, occupy the same region of cortex as commissural afferents from the contralateral DM and are positioned to intersect both layer 1 cells and the ascending dendrites of layer 2 cells. Some of the axons proceed through DM and reach layers 2 and 3 in the medial part of dorsal cortex. The projection reaches the full rostrocaudal extent of DM. Brainstem afferents are thick, smooth axons which do not resemble in their morphology the catecholaminergic fibers described in the cortex of turtles (Parent and Poitras, 1974, Brain Res., 78: 345) and lizards (Baumgarten and Braak, 1968, Z. Zellforsch., 86: 574). The precise origin of these cortical afferents is, unfortunately, not yet known. (Supported by PHS Grant NS 12518).

219 LIGHT SENSITIVE NEURONS IN THE INFERIOR PARIETAL CORTEX OF THE RHESUS MONKEY. Tom C.T. Yin and Vernon B. Mountcastle. Dept. Physiology, Johns Hopkins University, Baltimore, MD. 21205

Studies of single neurons in the parietal cortex of alert monkeys suggest that there exists within area 7 a neural mechanism for the direction of visual attention. Classes of visuomotor cells related to fixation, visually-evoked saccades, and smooth pursuit eye movements were identified. These cells are neither sensory nor motor in the usual sense. We have now studied a new class of neurons in area 7 which is sensitive to visual stimuli. They have large, contralateral receptive fields which sometimes extend past the midline but never include the fovea. Two subclasses have some functional properties that differ. Those of the larger subclass discharge at shorter latencies (79 $\pm$ 6 msec), subtend larger receptive fields with maximal activity evoked by stimuli at their peripheral rims, and discharge tonically during maintained visual stimulation; their discharge is not enhanced when the animal saccades to the stimulating light. Those of the smaller subclass respond at longer and more variable latencies (116 $\pm$ 26 msec), may subtend smaller receptive fields, and respond to the onset of a maintained visual stimulus with a phasic discharge which may be enhanced if the animal saccades to the test light. Cells of either subclass are sensitive to moving stimuli and may be directionally sensitive as well.

Light sensitive parietal cells differ from the saccade visuomotor cells: (1) the saccade cells do not respond to our test light (a light-emitting diode, λ = 660 nm); (2) many saccade cells discharge differently when saccades of the same amplitude and direction are made in different positions in the field. Visual fixation cells are not sensitive to the light stimuli, per se, for over 90% of them have restricted gaze fields; i.e. they are activated only when the animal fixates the light in a restricted zone of the visual field.

Saccade cells discharge, on average, 126 $\pm$ 39 msec after light onset and 73 $\pm$ 39 msec before eye movement. Thus, the light sensitive cells discharge well-before the saccade cells, which suggests that the former may provide the afferent input signals to the visuomotor neurons of area 7. Moreover, they are maximally sensitive to the appearance of novel objects in the far peripheral edges of the visual fields. Their properties suggest that the light sensitive cells are driven by signals reaching them via the retino-collicular branch of the visual system.

CHEMICAL SENSES: SMELL AND TASTE

220 THE FLAVOR CHEMISTRY OF CAT FUNGIFORM PAPILLAE TASTE SYSTEMS. James C. Boudreau and Joseph Oravec*. Sensory Sciences Center, University of Texas at Houston, 77030.

The properties of fungiform papillae taste systems were investigated by recording single unit pulse potentials from single neurons in the geniculate ganglion. These neurons were found to be responsive to a wide variety of water soluble compounds naturally present in vertebrate tissues, such as inorganic ions and a wide variety of nitrogen and nitrogen-sulfur compounds, including nucleotides. Among the salts, phosphate compounds and $CaCl_2$ are particularly stimulatory. Certain amino acids (L-cysteine, L-proline, L-lysine, L-histidine) and peptides (e.g., carnosine), are highly stimulatory as are the di- and tri- phosphate nucleotides. Nitrogen heterocyclic rings with pKa values greater than 5.0 are often associated with prominent stimuli. Unresponsiveness or inhibition is associated with anionic groups, aromaticity hydrophobicity, amine groups with low pKa values and most non-acidic oxygen compounds. The chemoresponsive neurons of the geniculate ganglion are divisible into three distinct functional groups with differing fiber diameters. These groups are selectively responsive to different chemical aspects of food solutions, with two of the unit groups being highly responsive to selected nitrogen compounds. Both unit groups I and II maximally respond to 5 and 6 member ring N heterocycles, but their response could be differentiated in terms of the Pka of the active group and the effect of ring substituents. Group III unit stimuli are less well defined but large responses may be obtained to nucleotides at concentration levels at or below those commonly found in animal tissues. This work supported in part by NSF Research Grants.

221 LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF ACETYLCHOLINESTERASE IN THE MAIN AND ACCESSORY OLFACTORY BULBS OF THE MOUSE. Gail D. Burd*, Keith A. Carson and Jacob S. Hanker (Spon: A. Rustioni) The Dental Research Center and Neurobiology Program, University of North Carolina, Chapel Hill, N.C. 27514.

Light microscopic enzyme histochemical and electron microscopic morphological studies have previously demonstrated both the discrete laminar organization of the acetylcholinesterase (AChE)-positive fibers and the ultrastructural features of the layers of the main olfactory bulb (MOB). Our studies utilized catalytic osmiophilic polymer generation to demonstrate the fine structural localization of AChE in both olfactory bulbs.

In light microscopic studies of the MOB, dense areas of stained fibers and terminals were observed adjacent to the mitral cell layer (MCL) and in the periglomerular region (PGR). Sparse fiber staining was present in the granule cell layer (GCL). A band of stained fibers ran in the plane of section (coronal) subjacent and concentric to the MCL. Groups of stained fibers were also observed near the apical pole of the cells of the MCL. In the PGR densely stained fibers outlined individual glomeruli. Throughout the MOB, cell body staining was negligible. Ultrastructurally, stained fibers in all locations had similar morphology. They were small, about 0.5μ, and unmyelinated. The stain was located on the axolemma and in the adjacent extracellular space. Frequently larger processes, presumably dendrites, apposed to the stained fibers also had stained plasma membranes. In the GCL, stained fibers appeared among groups of unstained fibers as well as near granule cell perikarya. Groups of stained fibers in the external plexiform layer(EPL) were observed near proximal dendrites. Profiles of stained fibers circumscribed individual glomeruli and, often, stained fibers were closely associated with nerve cells in the PGR. Stained fibers did not appear to be localized near one specific nerve cell type, but instead they were in close proximity to all three types.

The accessory olfactory bulb (AOB) had a different staining pattern. In contrast to the MOB, the glomerular layer of the AOB contained few, if any, AChE-positive fibers. The EPL had a small number of stained fibers, while many more were found subjacent to the MCL and in the GCL. Ultrastructurally, the stained fibers were very similar to those of the MOB.

The absence of AChE-positive neurons in the MOB and AOB indicates that the stained processes are extrinsic afferents. AChE positive perikarya have been found in the horizontal limb of the nucleus of the diagonal band, far lateral preoptic area, rostral lateral hypothalamus and the corticomedial complex of the amygdala. These areas have been suggested to contribute some of the centrifugal fibers to the olfactory bulbs. Supported by NIH grants DE 02668, DE 00288, MH 14277, HL 15888 and RR 05333.

222 ENHANCEMENT OF TASTE RECEPTOR BINDING BY EXPOSURE TO HIGH STIMULUS CONCENTRATION. Robert H. Cagan, Veterans Administration Hosp., and Monell Chemical Senses Center, U. of Pa., Philadelphia, PA 19104

A sedimentable fraction (P2) containing plasma membranes from the taste epithelium of catfish (*Ictalurus punctatus*) barbels was shown to bind L-[^{3}H]alanine [Krueger and Cagan, J. Biol. Chem. 251: 88-97 (1976)]. L-Alanine is among the amino acids shown electrophysiologically to be stimulatory to the catfish taste system [Caprio, Comp. Bioch. Physiol. 52A: 247-251 (1975)]. Our previous procedure, which employed the taste epithelium from only the barbels, has been extended in the present work to include the taste epithelium from the rostral, dorsal and dorsolateral surfaces of the fish. Previously this was difficult because of excessive mucus secreted on the body surface. It is now made possible either by intramuscular injection of atropine or by rapid sacrifice of the fish, which helps avoid the mucus contamination. Alanine-binding activity is enriched about 10-fold in Fraction P2 compared with the whole homogenate. Recovery of the binding activity in this fraction is essentially quantitative. By using the additional taste tissue noted as well as that of barbels, the yield (mg protein) of Fraction P2 is increased by around 10-fold.

The present results show an increase in the amount of L-[^{3}H] alanine bound to Fraction P2 after its prior exposure *in vitro* to a high concentration of L-alanine. Previously we noted (Krueger and Cagan, *op. cit.*) the loss of binding activity of Fraction P2 upon storage at -65°C. It is now demonstrated that similar storage of Fraction P2 in a high concentration of ligand (10 mM L-alanine, unlabeled) results in a substantial increase of binding (5 to 7-fold). Binding is measured after removal of the storage L-alanine by centrifugation and washing Fraction P2 twice with buffer. The enhancement is not contingent upon freezing the preparation; it occurs, for example, by exposure to 10 mM L-alanine for 30 min on ice. Prior exposure to D-alanine causes enhancement but to a lesser extent; it is also a less effective electrophysiological stimulus (Caprio, *op. cit.*).

The results could indicate the presence of "hidden" receptor sites, possibly as a consequence of the isolation procedure or possibly existing *in vivo*. It is postulated that some of the receptor macromolecules are readily accessible to ligand and others are localized within the cell membrane such that they become accessible upon perturbation of the membrane structure due to a high ligand concentration. (Supported in part by NIH Research Grant NS-08775 from NINCDS.)

223 RESPONSES OF HAMSTER RECEPTOR NEURONS TO CONTROLLED ODOR STIMULATION. Richard M. Costanzo and Robert J. O'Connell*. The Rockefeller University, New York, N. Y. 10021.

Selection of appropriate test odorants and precise control over their purity, concentration, and waveform are critical components to an adequate study of quality coding in the olfactory system. Although it is usually assumed that most mammals respond to a range of different odor qualities, the selection of a particular test odorant is often arbitrary and unrelated to those compounds normally discriminated by the experimental animal. Recent work in this laboratory has led to the isolation and identification of several biological odorants from estrous hamster vaginal discharge. This material contains compounds which normally elicit strong behavioral responses in the male (pheromones). Since the olfactory input for these behaviors must be initially processed by the receptors, it is conceivable that at least some receptor neurons might be more selective in their response to behaviorally active compounds. Seven compounds (chemical purity $>$ 99%) were selected for study. Three of these, n-amyl acetate, ethyl acetate, and ethyl isobutyrate, are traditional odors not found in vaginal discharge. The remaining compounds, dimethyl disulfide, n-butanol, n-hexanol, and ethyl n-butyrate, are found in vaginal discharge; and one, dimethyl disulfide, is a powerful behavioral attractant for the male. An improved flow dilution olfactometer was constructed which included high efficiency odor saturators (teflon packed glass columns), a teflon mixing chamber, and a rotary sample valve for on-line injection of odor stimuli. Quantitative calibrations of both the stimulus concentrations and waveforms produced by the olfactometer were made using a flame ionization detector.

Odor stimuli were delivered to the exposed olfactory mucosa and extracellular responses recorded with metal microelectrodes. Most of the units studied to date are responsive to all odorants; however, the relative sensitivity to these compounds varies from neuron to neuron. The concentration-response functions for a typical odorant usually encompass 1-2 log units of concentration while individual thresholds for different compounds range from 3.16×10^{-7} to 1.0×10^{-4} molar. We have yet to observe a neuron exclusively sensitive to one odor. (Supported by NIH Grant NS 08902.)

224 EFFERENTS AND CENTRIFUGAL AFFERENTS OF THE HAMSTER OLFACTORY BULB. B.Davis, F.Macrides, W.Youngs* and S.Schneider*. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The efferents and centrifugal afferents of the main olfactory bulb (MOB) were studied in the hamster using orthograde and retrograde tracing techniques. Following injections of tritiated amino acids which were restricted to the MOB, autoradiographic grains were observed ipsilaterally in layer IA of the entire anterior olfactory nucleus (AON), the so-called anterior continuation of the hippocampal rudiment, the entire rostrocaudal extent of the prepyriform cortex and olfactory tubercle, the anterior and posterolateral cortical amygdaloid nuclei and the lateral entorhinal cortex. No subcortical or contralateral projections were observed. Following injections of HRP which were restricted to the MOB, contralateral HRP-positive cells were identified only in pars externa, dorsalis, lateralis, ventralis and posterior of the AON. More heavy labelling and a wider distribution of labelled cells were observed ipsilateral to the injected MOB. HRP-positive cells were found throughout the AON, in the anterior continuation of the hippocampal rudiment and in the anterior portion of the prepyriform cortex. No labelled cells were found in the olfactory tubercle, cortical amygdaloid nuclei or entorhinal cortex. HRP-positive cells were also observed ipsilaterally in subcortical structures of the basal forebrain and bilaterally in the midbrain. Labelling was observed in the fusiform cells of the diagonal band near the medial base of the forebrain at the level of caudal olfactory tubercle. Heavy labelling was seen in a distinct group of large, predominantly multipolar cells ("nucleus of the horizontal limb of the diagonal band" or "magnocellular preoptic area") that continued from the level of caudal olfactory tubercle to the level of the nucleus of the lateral olfactory tract. This band of HRP-positive cells could be followed more caudally to a position dorsal and medial to the nucleus of the lateral olfactory tract at the lateral margin of the lateral anterior hypothalamic area. Well-defined, isolated cells were found in the dorsal and median raphe nuclei, and on rare occasions in the locus coeruleus. The finding that the anterior continuation of the hippocampal rudiment is reciprocally connected with the ipsilateral MOB suggests that it may be grouped with the AON and anterior prepyriform cortex as structures which exert cortico-cortical influences on MOB function. The observed projections from the subcortical forebrain and midbrain may mediate limbic-hypothalamic influences on MOB function. (Supported by NINCDS grant NS12344 and NSF grant BNS75-07652)

225 CONDITIONED TASTE AVERSION IN GENETIC OBESITY. Melvin Enns*, Geoffrey Nowlis*, Joel Grinker* (Spon: Victoria Luine). Rockefeller University, New York, New York 10021.

Sucrose consumption of 24 genetically obese and 24 lean female Zucker rats (age 3-4 mo., wt. $\bar{x}$ = 432g and $\bar{x}$ = 220g) was examined using a conditioned taste aversion paradigm. Rats received either .1M sucrose (experimental) or water (control) paired with apomorphine hydrochloride (30mg/kg ip) and then the intake of .01M, .0316M, .1M and .316M sucrose was measured. The degree of the conditioned aversion was similar for obese and lean rats and was a function of test solution concentration; experimental subjects drank significantly less .1M and .316M sucrose than controls. Following a second conditioning trial, the intake of .75M glucose, .1M sucrose, .001M saccharin and .025M sodium cyclamate was measured. Again, the degree of the conditioned aversion was similar for obese and lean rats and was related to type of sweetener; experimental subjects drank significantly less .75M glucose and .1M sucrose than controls. Next, .1M sodium chloride was paired with the apomorphine hydrochloride and the intake of .1M sodium chloride was measured. The degree of the conditioned aversion and the rate of extinction were similar for obese and lean rats; experimental subjects drank significantly less .1M sodium chloride than controls on the first test day but not on days 2,3 and 4.

In another experiment, the same absolute dose of apomorphine hydrochloride for all subjects (equivalent to 30mg/kg for lean rats) produced similar conditioned taste aversions between 16 obese and 16 lean female Zucker rats (age 4-6 mo.; wt. $\bar{x}$ = 445g and 224g) to .1M sucrose or .1M sodium chloride. However, during four weeks of weekly conditioning and daily testing, experimental and control obese rats decreased their sucrose intakes while experimental and control lean rats increased their sucrose intakes. These long-term changes did not occur with sodium chloride. The data confirm previous findings of normal or subnormal sucrose intakes by obese Zucker rats (Grinker and Roswell, EPA, 1974; Grinker, Obesity in Perspective, 2, 1975) and suggest that experiential rather than sensory factors contribute to the decreased intake. (This work is supported by NSF Grant BNS 76-09957).

226 TRANSMISSION WITHIN OLFACTORY RECEPTORS. R. C. Gesteland, J. A. Weier*, G. D. Adamek* and R. A. Yancey*. Northwestern Univ. Dept. of Biol. Sci., Evanston, IL 60201.

Current pulses injected intracellularly in mud puppy olfactory receptor neurons evoke action potentials when they depolarize the soma membrane as little as 1 mv. These neurons, like olfactory receptors in other vertebrates, fire spontaneously and irregularly in the absence of stimulation. Low intensity odorous stimulation commonly acts by adding additional impulses without changing the slow burst patterns of the spontaneous activity. Thus these cells apparently achieve high sensitivity by being poised close to a noisy threshold. It would be expected that more intense stimulation would evoke large depolarizations and a regular-interval, rapid action potential rate. However, intracellular records from the soma region, the only portion of the cell large enough to sustain penetration, do not support this. The maximum depolarization recorded with highest achievable stimulus concentrations is a few mv. Most stimuli presented at intensities sufficient to increase spike rate evoke soma depolarizations too small to be distinguished from noise. Further, while penetration-evoked soma depolarization results in action potentials of 60 mv, naturally-occurring ones never have amplitudes greater than 10 mv. Thus action potentials do not invade the soma actively, stopping 2 or more length constants away. Since the soma lies between a long dendrite at the end of which the stimulus acts and the axon, the receptor region is separated from the site of spike origin by 4 or more length constants. The intervening soma effectively decouples the dendritic receptor potential from the axonal generator potential and both from the recording electrode. The longitudinal current flow in the cell transmits the stimulus information and this current cannot be measured directly. We would expect considerable loss of current through the unsheathed soma and axon membranes and, as a result, poor sensitivity of the receptors. Extracellular measurements suggest that an unusual transmission process which avoids this loss may be involved. Monopolarly-recorded action potentials of single cells in the soma region commonly have 6 or 7 clearly distinguishable phases. These could result from an abrupt change in propogation velocity of the action potential in the vicinity of the recording electrode or from summation of more than one active event. The former is not likely here since the action potentials do not invade the soma. It may be that the stimulus evokes dendritic action potentials and that current from these modulates the rate of occurrence of action potentials of axonal origin. Supported by NSF Grant No. BNS 75-02339 and Northwestern University.

227 CHOLINE ACETYLTRANSFERASE AND ACETYLCHOLINESTERASE IN THE OLFACTORY SYSTEM OF THE RAT. D.A. Godfrey*, C.D. Ross* and A.D. Williams* (SPON: N.R. West). Depts. of Pharmacol. and Anat. and Neurobiol., Washington Univ. Med. Sch., St. Louis, Mo. 63110.

Quantitative histochemical mapping procedures have been applied to measure choline acetyltransferase (ChAc) and acetylcholinesterase (AChE) activities in the olfactory system of male Sprague-Dawley rats. Data for many regions in one rat, which have been supported by measurements on certain of these regions in three other rats, are summarized below, enzyme activities in amount/kg dry wt/min, mean ± SEM (no. of samples):

Region	ChAc(μmoles)	AChE(mmoles)
OLF. BULB, olf. nerve layer	9±3(3)	2±1(6)
glomerular layer	472±40(6)	42±3(11)
ext. plexiform layer, superf.	557(2)	48(2)
ext. plexiform layer, deep	353±20(6)	30±1(8)
mitral cell body layer	408±30(3)	37±1(3)
int. plexiform layer	439±24(3)	42±2(6)
granular layer	224±14(11)	25±2(10)
ANT. OLF. NUCLEUS, layer I	328±21(14)	33±1(15)
layer II	349±11(17)	39±1(27)
ant. commissure	52±22(3)	10±1(4)
PIRIFORM CORTEX,ROSTRAL, layer I	271±35(4)	29±2(8)
layer II	558±34(3)	50±3(4)
layer III	447±57(4)	54±5(7)
PIRIFORM CORTEX,CAUDAL, layer I	510±67(5)	60±9(4)
layer II	1115(2)	119(2)
layer III	1264±235(3)	165±28(5)
OLF. TUBERCLE, layer I	1230±80(3)	307±26(4)
layer II	1450(2)	480±39(3)
layer III	1362±79(10)	437±33(9)
NUCLEUS OF LOT, layer I	964±34(3)	116±16(9)
layer II	2563±139(3)	258±12(8)
layer III	1428±242(4)	151±34(3)
HORIZ. LIMB OF DIAGONAL BAND	982±55(10)	149±4(6)
LATERAL OLF. TRACT (LOT)	70±13(12)	8±1(11)

These data suggest that the olfactory nerve fibers and the mitral cells (whose axons comprise the bulk of the lateral olfactory tract) are not cholinergic, but that cholinergic neurons are nevertheless prominent in the olfactory system. Attempts to identify these cholinergic neurons have been started in collaboration with Dr. J. L. Price, through measurements of enzyme activities following surgical lesions at various locations. (Supported by NIH Grant NS08000 to F. M. Matschinsky).

228 IDENTIFICATION OF GENICULATE GANGLION SOMATA THAT PROJECT VIA THE RAT CHORDA TYMPANI USING HORSERADISH PEROXIDASE. Maximo M. Gomez. Dept. Anat., Bowman Gray Sch. Med., Winston-Salem, N.C. 27103

The chorda tympani nerve contains chemosensitive and mechanosensitive fibers whose cell bodies are located within the geniculate ganglion (GG). This study was designed to determine the topographic organization of these somata within the GG and to distinguish them from the neurons whose peripheral processes run in the greater superficial petrosal (GSP) and posterior auricular (PA) nerves. The CT was cut in the infratemporal fossa, several dry flakes of horseradish peroxidase were applied to the central end three times during an 8 hr. period and allowed to disolve in the surrounding tissue fluid. After intracardiac perfusion and fixation, both the experimental and contralateral control GG were removed and reacted en bloc with diaminobenzidine and H_2O_2, dehydrated, embedded in paraffin and serial sectioned at 15u. In the experimental GG, 3% of the total GG cells contained HRP positive granules. These cells are all in the lower size range of GG somata, being 15u to 25u in maximal cross-sectional diameter. The labeled cells are primarily located in the ventral region of the GG where they are spread close to the main trunk of the VIIth nerve (posterior in the ganglion). The labeled cells that are found more dorsally within the GG nearly always occur along the medial margin of the ganglion. Although these preliminary results are not quantitative for the afferents in the CT, similar HRP experiments are in progress to determine the subpopulations of somata within the GG that project via the GSP and PA nerves. This will allow the study of the architectonics of these neuronal subpopulations and a correlation between some aspect of perikarial morphology or location and the peripheral sensory field to which they project.

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229 TOPOGRAPHIC ORGANIZATION OF THE ASSOCIATION FIBER SYSTEMS IN THE OLFACTORY CORTEX OF THE RAT. L.B. Haberly*, M.B. Luskin*, and J.L. Price (Spon: M.B. Bunge). Dept. Anat. and Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

Studies of the association fiber systems in the olfactory cortex of the rat, using HRP as a retrograde and anterograde axonal tracer and the autoradiographic method, have revealed the presence of a broad and overlapping, but systematic topographic organization. This organization is present in the projections to the cortical areas at the level of the lateral olfactory tract (anterior olfactory nucleus, anterior piriform cortex, olfactory tubercle, and ventral agranular insular cortex) and encompasses the projections from the anterior olfactory nucleus, piriform cortex, and a newly discovered projection from the entorhinal cortex. These fiber systems are organized such that fibers from progressively more caudal areas terminate in broad strips, oriented parallel to the lateral olfactory tract, which are progressively further removed from the tract on both the lateral and medial sides. Thus the anterior olfactory nucleus (the most rostral olfactory cortical area) projects most heavily to the cortex deep to the tract; the anterior piriform cortex projects to the cortex deep and adjacent to the tract both medially and laterally; the posterior piriform cortex projects to strips further removed from the tract with little projection to the cortex deep to the tract; the entorhinal cortex projects only to the cortical areas furthest removed from the tract (most medial portions of the olfactory tubercle and the anterior olfactory nucleus).

There is also an organization in the tangential dimension in other portions of the association fiber system, but no other clear examples of topographically ordered (systematic point to point) projections have been found. Thus while many of the olfactory cortical areas or cytoarchitectonically distinct subdivisions of these areas have characteristic projection patterns to restricted regions, neighboring areas often project to non-adjacent regions.

Commissural fiber projections from three cortical areas have been demonstrated (from the anterior olfactory nucleus to the opposite anterior piriform cortex; from the anterior piriform cortex to the opposite posterior piriform cortex and adjacent cortical areas; from the nucleus of the lateral olfactory tract to the medial portion of the opposite anterior piriform cortex and adjacent portion of the olfactory tubercle). In all cases the commissural projection is matched by a very similar ipsilateral projection from the same area, but no evidence for any further degree of organization of a topographic nature has been found within any of these systems. Supported by NIH Grants NS09518 and NS05162.

230 BEHAVIORAL AND BIOCHEMICAL SUPPORT OF OLFACTORY NEURON REPLACEMENT. Joseph W. Harding[1], Kate Donlan[2*], Nancy Chen[1*], John Wright[2*]. Dept. Veterinary Pathology, Dept. Pyschology[2], Washington State Univ., Pullman, WA 99164.

Behavioral and biochemical evidence is presented which strongly suggests that the primary olfactory chemoreceptor neurons in the adult mouse are capable of being replaced, presumably from a population of undifferentiated stem cells. This data further corraborates previously published biochemical and morphological studies which supported the unusual notion of neuron replacement in adult mice (Harding et al, Brain Res, in press). Mice were trained to find buried food pellets of various sizes within a time period of three minutes. After training, mice were bilaterally olfactory nerve sectioned. Starting two days after nerve section and continuing until 30 days postop, the olfactory capabilities of the animals were determined using the food finding test. At day 3 a few operated animals began to find the buried food pellets. The number of animals finding the buried pellets steadily increased and by day 19 all the operated animals were finding the pellets. Although all the operated animals were finding the pellets, the latency for finding was longer than for unoperated mice. Animals were taken for biochemical analysis at various points during the time course of loss and return of olfactory mediated behavior. At days 3,9,12,21, and 45, mice were intranasally irrigated with ^{14}C-β-alanine, the precursor of the specific olfactory neuron marker, carnosine. Carnosine is known to be synthesized in the olfactory epithelium and transported to the bulb. Both ^{14}C-β-alanine and ^{14}C-carnosine content were measured in the olfactory epithelium and olfactory bulb. Three days following nerve section the amount of ^{14}C-carnosine transported to the bulb was less than 10% of control values. The amount transported increased during the time course of the experiment and by day 45 had reached approximately 55% of control values. This return in the ability of the primary olfactory system to synthesize and transport the neuron specific marker, carnosine, is consistent with the return of olfactory mediated behavior, the concept of neuron replacement, and the formation of new functioning synapses with the bulb.

231 PREPARATION AND CHARACTERIZATION OF CELLS FROM RAT OLFACTORY MUCOSA. James D. Hirsch* and Frank L. Margolis. Dept. Physiol. Chem. & Pharmacology. Roche Inst. of Molec. Biol., Nutley, NJ

Our lack of understanding of olfaction may be blamed in part on the inability to assess the functions of the individual cell types in the olfactory mucosa. Interaction of odorant molecules with the chemosensory neurons is known to occur but this process is poorly understood. The role of the sustentacular cells is unclear. The basal cells are progenitor cells for the neurons, but the factors controlling their division and differentation are unknown. Thus, studies of isolated olfactory cells should be helpful to elucidate the individual cellular functions in olfaction.

The turbinates and nasal septum containing the olfactory and respiratory epithelia were subjected to enzymatic digestion with collagenase and hyaluronidase and mechanical disruption yielding a suspension of viable cells which synthesize RNA and protein from radiolabeled precursors. The olfactory neurons, sustentacular cells and respiratory epithelial cells could be identified by phase contrast microscopy, but the basal cells could not be identified. The cell suspensions were fractionated in discontinuous gradients of bovine serum albumin at unit gravity. Separation of the olfactory neurons from the various non-neuronal olfactory cells was achieved. Cells containing the olfactory neuron-specific marker protein (OMP) and the neuronal enzyme carnosine synthetase were in the upper fractions of the gradients. Nonneuronal cells containing carnosinase were in the lower fractions. These gradients were also analysed by immunocytochemical and histochemical techniques. The OMP and S-100 proteins were localized in cells from the gradient by staining with the PAP technique. S-100-containing nonneuronal cells were partially separated from OMP containing neurons by our fractionation technique. This is the first report of the presence of the S-100 protein (a presumptive glial marker) in this tissue and raises questions as to which olfactory cell type contains this protein and whether they are glial-like. Staining for secretory cells of Bowman's glands with Alcian Blue 8GS (pH 1.0), showed their presence in the suspension and their separation from the neurons. The Bowman's gland cells sedimented rapidly in the gradient.

Clearly, care must be taken to characterize separated cells by a variety of morphological and biochemical criteria before conclusions are drawn about the purity of the cell fractions. We have obtained cell suspensions enriched in olfactory neurons or nonneuronal cells. Work is in progress to purify these fractions further. The cell fractions will be used to probe the role of the individual olfactory cells in olfaction and study the interaction of odorant molecules with the chemosensory neurons in vitro.

232 SINGLE UNIT ACTIVITY IN THE OLFACTORY PATHWAY IN THE PIGEON. Larry V. Hutchison, Lyle J. Rausch and Bernice M. Wenzel. Dept. Physiol., UCLA Sch. of Med., Los Angeles, CA 90024.

Previous evoked potential and neuroanatomical studies in our laboratory identified 3 structures in the pigeon forebrain as primary ipsilateral projection areas of the olfactory bulb (OB), viz., cortex prepiriformis (CPP), lobus parolfactorius (LPO), and hyperstriatum ventrale (HV). Also identified were several secondary projection areas, viz., hyperstriatum accessorium (HA), hyperstriatum dorsale (HD), paleostriatum augmentatum (PA), paleostriatum primitivum (PP), nucleus septalis laberalis (SL), and neostriatum caudale (NC). We now report results of electrically stimulating the pigeon's olfactory nerve (ON) and recording extracellular single unit responses in the OB and ipsilateral projection areas confirming previous evoked potential records. Mean spike discharge rates in forebrain projection areas varied over a wide range for different cells (0.28->30 spikes/sec). Stimulation of ON with 8-12 V pulses (single or train), 0.5-1.0 ms duration, at 0.1-1.0 Hz resulted in modification of spontaneous activity (+25% change in rate). Response patterns of the majority of affected cells involved enhancement while a smaller number of cells showed depression. PST histograms for some cells showed peak spike discharge rates or periods of inhibition at a constant latency, consistent with latencies of phases of the evoked potential characteristically recorded from that site or through the same electrode. There appeared to be some degree of anatomical localization of effects; LPO and PP show a larger proportion of cells inhibited by ON stimulation, whereas the cell populations of the projection areas of the hyperstriatal complex generally show excitation. Records to date indicate that low rates of spontaneous activity may be characteristic of the mitral cell layer of the OB and in some cases a burst of spikes may be elicited at a latency comparable to the bulbar response (20-40 ms).

Tetanic stimulation of ON (20 Hz-20 sec) produced either potentiation-recovery or depression-rebound-recovery in forebrain projection sites. The time course of post-tetanic changes varied markedly for individual units from a few seconds to several minutes. Active units in other forebrain areas not known to receive olfactory input were not effectively modified by ON stimulation. (Supported by USPHS grant NS 10353 to B.M. Wenzel and a postdoctoral fellowship to L.V. Hutchison under grant MH 06415 to the UCLA Brain Research Institute.)

233 RESPONSE OF THE HAMSTER CHORDA TYMPANI NERVE TO TWO-COMPONENT MIXTURES. Angela M. Hyman* and Marion Frank* (SPON: C. Pfaffmann). Rockefeller Univ., New York, N. Y. 10021.

Most mammals are stimulated by taste mixtures in natural feeding situations. Yet, neurophysiological investigations have principally considered single-component stimuli. For instance, although the hamster chorda tympani nerve has been well characterized in its responses to individual substances, its responses to mixtures have not previously been studied.

Summated electrophysiological responses of the whole nerve were recorded to .01M NaCl, .05M NH_4Cl, .01M $CaCl_2$, .003M HCl, .1M sucrose, and .07M D-phenylalanine applied to the tongue individually and in two-component mixtures. The actual response to each mixture was less than the value calculated as the arithmetic sum of the responses to the components presented alone.

The response to a mixture will equal the value calculated as the arithmetic sum of the responses to the individual components if the components stimulate two independent sets of receptors. Alternatively, if the components stimulate the same set of receptors, the response to a mixture can be predicted from the response functions of either component presented alone. The response to a mixture will lie between these extremes if the sets of receptors stimulated by the components partially overlap. In order to determine which of these alternatives best describe the mixture data, summated responses were also recorded to the individual substances in ascending concentration series. Power functions were fitted to the response values for each substance at its concentration in the mixtures and plus and minus ½ log-step. The response to each mixture could then be scaled between the theoretical value predicted from the response functions and that calculated as the arithmetic sum of the component responses. Values on this scale express the degree of overlap of receptor stimulation by the different substances. The scaled values for the different mixtures indicate, for example, that $CaCl_2$ and HCl predominantly stimulate the same set of receptors whereas NaCl and D-phe do not. The individual stimuli can be divided into two groups: sucrose and D-phe, and the electrolytes. Although the extent of the overlap between sets of stimulated receptors varies, in general, it is greater between substances in the same group than between substances in the different groups. However, the electrolytes overlap somewhat more with sucrose than with D-phe.

Inhibition was observed when certain electrolytes (e.g., $CaCl_2$ and NH_4Cl) were mixed at these concentrations.

234 OBSERVATIONS ON MORPHOLOGICAL CHANGES IN THE VOMERONASAL ORGAN OF GARTER SNAKES FOLLOWING VOMERONASAL NERVE SECTION. John L. Kubie*, Ruu Tong Wang* and Mimi Halpern (Spon: K. Koizumi). Dept. of Anatomy and Cell Biology and Program in Biological Psychology Downstate Medical Center, Brooklyn, N.Y. 11203.

Following vomeronasal (VN) nerve section the VN mucosa of garter snakes undergoes a dramatic retrograde necrosis which when viewed in Bodian stained sections is marked by a loss of the cell bodies occupying the bipolar nuclear layer, a loss of the dendrites of bipolar neurons and an apparent thinning of the superficial epithelial layer. The layer of undifferentiated cells does not undergo major morphological changes.

Partial VN nerve section results in degeneration of bipolar cells in those columns from which the severed axons emerged. Results of several partial vomeronasal nerve lesions reveal that the projection from the VN organ to the accessory olfactory bulb via the VN nerve is topographically organized: dorsally situated VN nerve fibers originate from bipolar neurons in the anterior, dorsal portion of the organ; ventrally situated fibers originate from caudal, ventral regions of the organ.

Electron microscopic observations of the VN organ two weeks after transection reveal that although the columnar cells themselves do not undergo degeneration, the organization of the epithelial cell layer changes from a simple columnar type with cell nuclei restricted to its basal portion to an epithelium with many nuclei lying adjacent to the luminal surface. Thinning of the epithelial layer is partially due to shortening of columnar cell cytoplasmic processes which line the lumen of the organ. In addition, following nerve cuts, columnar cells contain an increased number of lysosomal and secretory granules particularly at their luminal pole.

In the bipolar cell layer a marked loss of cell bodies is observed. Cells undergoing degeneration become polymorphic, contain many large lysosomes, multivescicular bodies and lamellar vescicles. Cytoplasmic organelles become disorganized. The nuclei are reduced in size, irregular in shape and contain randomly dispersed chromatin. Intermingled with the debris of degenerated neurons were a few neurons whose morphological characteristics were identical to normal bipolar neurons. In addition, at the base of the bipolar nuclear layer 2 or 3 compact rows of neuron-like cells were observed. We presume that the remaining neurons are either cells which differentiated following the nerve cuts or cells whose growing axons had not yet reached the level of the nerve cut at the time of surgery.

The layer containing undifferentiated cells was free of signs of degeneration. Degenerating columns were marked by cell proliferation and an increased frequency of mitotic figures when compared with healthy columns in the same animal. Supported by NIH grant NS 12152

235 ORBITOFRONTAL NEOCORTEX OF RATS AND ODOR DISCRIMINATION. L. Leach*, S. Kiefer*. Ariz. St. Univ., Dept. of Psych., Tempe, AZ 85281. (SPON: E. Davis, Dept. of Anatomy, Univ of Calif. at Irvine, Medical School, Irvine, CA 92717).

Leonard (Brain, Behav, Evol. 6: 524, 1972) has shown that the rat's orbitofrontal neocortex receives input from the primary olfactory cortex (piriform area) via the dorsomedial thalamic nuclei. The present study compared the ability of unoperated rats (Group U, n=20) and rats with bilateral orbitofrontal ablations (Group OF, n=10) to acquire a discriminative learned odor aversion. Three stimulus conditions were used. In condition PO drinking water in the presence of an odor was followed by toxicosis induced by injections of apomorphine hydrochloride. In condition UO drinking water in the presence of an odor different from that used in condition PO was never paired with toxicosis. In condition NO all rats drank water in the absence of an odor without the induction of toxicosis. It was found that bilateral orbitofrontal ablations impaired the ability of rats to associate odors with toxicosis and/or to discriminate between the paired and unpaired odors. After 6 presentations of each condition, rats in Group U exhibited the ability to discriminate between paired and unpaired odors. The Group U rats exhibited a significant aversion to drinking water during condition PO relative to either conditions UO or NO, but did not exhibit a significant aversion to drinking water during condition UO relative to condition NO. After 6 presentations of each condition, rats in Group OF drank less water during both conditions PO and UO relative condition NO but the differences were not significant. It is concluded that in the rat the region of the orbitofrontal neocortex shown by Leonard (1972) to receive input lower olfactory areas via the dorsomedial thalamic nuclei plays a role in acquiring a disciminatively specific avoidance to odors.

236 A THEORETICAL MODEL OF CHEMOSENSORY TRANSDUCTION IN CATFISH. Joseph F. Metcalf. Center for Sensory Studies. University of Florida, Gainesville.

The olfactory and gustatory chemoreceptors of catfish are extremely sensitive to low stimulus concentrations, and show a high degree of specificity for amino acids. The stimulus-response relationship does not obey Beidler's fundamental taste equation, however, and is best described as a power function with exponents between 0.08 and 0.14 (Suzuki and Tucker, Comp. Biochem. and Physiol, 1971; Caprio, O. and T. V, 1975).

A simple modification of a previous model (Metcalf, Neuroscience Abstracts, 1976) is proposed to account for the rapidly adapting catfish chemoreceptor responses and predict the power function relationship between response and stimulus concentrations. The amino acid, A, is assumed to bind a specific receptor protein, P, which has a single binding site for the amino acid on the external surface of the receptor cell, and multiple (i) binding sites for substance X on the inner or cytoplasmic side of the membrane. X is assumed to be a precursor of substance Y. Binding of the amino acid to the active receptor protein P(X)i, produces an activated complex, A-P(X)i, which generates substance Y and the inactive receptor, A-P. Y is assumed to function as in internal transmitter, and the amplitude of the receptor response is assumed to be determined by the rate at which Y is generated. The active form of the receptor protein, P(X)i, is regenerated by subsequent steps of the reaction sequence.

The Continuous System Modeling Program (C.S.M.P.) was used with the IBM 370 computer to simulate the time-course of receptor response and generate response amplitudes as a function of stimulus concentration. The simulated responses show a rapid rise to a peak followed by a rapid decline to a low value. The response amplitudes are described by a power function of stimulus concentration extending over a wide range. The exponent of the power function, n, is equal to the reciprocal of i, the number of internal binding sites for substance X on the receptor molecule.

The proposed model is consistent with the time-course of receptor response, and accounts for the quantitative stimulus-response relationship, extended dynamic range, and extreme sensitivity of the olfactory and gustatory chemoreceptors in catfish.

237 TASTE RESPONSES TO SOLUTIONS OF DEUTERIUM OXIDE. Inglis J. Miller, Jr. and Gregory Mooser*, Dept. of Anatomy, Bowman Gray School of Medicine of Wake Forest Univ., Winston-Salem, NC 27103 and Dept. of Biochemistry, University of Southern California, Los Angeles, CA 90007.

Deuterium oxide (D_2O), the "heavy hydrogen" analog of water (H_2O), has been used in studies of excitable tissues because its chemical properties are similar to ordinary water but its physical properties differ because of greater atomic mass. Recently, Richter (P.S. E.B.M. 152, 677, 1976) demonstrated that rats discriminate between D_2O and H_2O in favor of water when offered a choice, but concluded that rats were unable to taste a difference among the two compounds. In the current study, summated electrophysiological responses were recorded from whole chorda tympani nerves in the rat in response to stimulation of the tongue with solutions of D_2O. Solutions consisted of sodium chloride, sodium benzoate, potassium chloride, potassium benzoate, quinine hydrochloride, and sucrose in concentrations from .01 to 1.0 molar for the salts. Sucrose concentrations included .02 to 2.0M and quinine HCl solutions of .003 to .03 M were used. Distilled H_2O was used to prepare control solutions of the same molar concentrations for comparison with solutions of D_2O. Application of D_2O to the tongue, which was previously rinsed with H_2O, resulted in an immediate response that was maintained until the D_2O was rinsed with H_2O. The magnitude of this response was intermediate to those elicited from H_2O solutions of NaCl between .03 and 1.0 M. NaCl and KCl responses from D_2O solutions were comparable in magnitude to H_2O solutions for concentrations above 0.1 M when this concentration was used as a standard; however responses to D_2O solutions of NaClat .01 and .03 M concentrations were greater in magnitude than for corresponding H_2O solutions. D_2O solutions of sucrose elicited responses which were smaller than equivalent concentrations of H_2O solutions; on the other hand, responses to quinine solutions of D_2O were greater than comparable H_2O solutions. Responses elicited when potassium benzoate was rinsed from the tongue with pure solvent, which have been called "water responses" by some investigators, could be elicited by D_2O. These preliminary observations are being continued to determine the effects of solvent-solute interactions on taste responses. (Supported in part by NIH Grant NS 10389).

238 INTERACTIONS BETWEEN NERVES AND EPITHELIA DURING TASTE BUD AND PAPILLA DEVELOPMENT IN FETAL SHEEP. Charlotte M. Mistretta and Robert M. Bradley. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

To study nerve-epithelium interactions during taste bud and papilla development, autogenous cross-grafts of anterior dorsal tongue mucosa and external cheek skin were made in 12 fetal sheep aged 52-107 days of gestation (term=147 days). Grafts, including epidermis and dermis, were made in fetuses during sterile surgical procedures; fetuses were then replaced in utero. At 134-144 days fetuses were delivered and fixed by perfusion. Graft and control tissues were dissected and prepared for light microscopy. In the youngest fetal age at the time of grafting (52 days, weight ~ 20 g) epithelium contains immature fungiform papillae and presumptive taste buds; cheek epidermis contains follicle plugs, the first stage in wool follicle development.

All grafts continued to differentiate at transplant sites and became innervated. Cheek skin transplanted to the dorsal tongue acquired the cornified cell layers, mature wool follicles, and wool fibers characteristic of normal mature cheek. Although grafts remained at the tongue site for 37 to 82 days, no fungiform papillae or taste buds formed. Tongue epithelium transplanted to the external cheek acquired additional cell layers and mature filiform papillae. However, only in tongue grafts that had been made in fetuses aged 52-67 days of gestation were there any structures resembling fungiform papillae or taste buds; in older fetuses the taste buds and papillae that were already present degenerated and did not re-form. About 1-5 fungiform papillae were present in each tongue graft made in the younger fetuses. These papillae had a typical fungiform shape and broad connective core, but were atypical in that they usually had a filiform-type, keratinized point on one edge. Two-three of these papillae on each graft contained a structure resembling an immature taste bud. The buds were composed of a cluster of disoriented cells with no taste pore.

From these initial results we conclude that: 1. Tongue nerve fibers will not induce taste bud and papilla development in nongustatory, cheek epidermis, even when the epidermis is grafted at a very immature stage of development. 2. Not only taste bud development, but also, fungiform papilla differentiation and maturation is under the influence of tongue nerves. 3. A few structures resembling taste buds and fungiform papillae can be induced to form in tongue epithelium under the influence of the nongustatory, trigeminal innervation to the cheek if the tongue is transplanted early enough in development. Therefore, it is suggested that nerves and epithelia interact differently at different stages of development in the process of taste bud and papilla differentiation. (N.I.H. Grant DE-04491)

239 DEOXYGLUCOSE AND ELECTROPHYSIOLOGICAL MAPPING OF CHORDA TYMPANI ACTIVITY IN RAT NUCLEUS SOLITARIUS. M.A. Orlandi*, R.P. Erickson, A.E. Johnson* and L.C. Skeen, Departments of Psychology, Physiology and Anatomy, Duke University, Durham, N.C. 27706

In order to better elucidate the taste pathways within the mammalian central nervous system, we have initiated a series of studies in rats which combine electrophysiological analyses with an autoradiographic method that allows anatomical localization of stimulus-induced cerebral deoxyglucose consumption (Science, 187: 850, '75). In the present studies, rats were given IV pulse injections of ^{14}[C]-2-deoxy-D-glucose (150-250μCi/kg) and exposed either to a) natural taste stimuli on the tongue (e.g., 0.1M NaCl), or b) electrical stimulation of chorda tympani nerve (3-15v/0.2ms., 20/sec) combined with recording electrode placements in nucleus tractus solitarius (NTS) guided by natural taste stimuli. The animals' brains, and those of control rats, were subsequently processed for x-ray film autoradiography (DuPont, LoDose). Under the latter conditions, maximal ipsilateral NTS activity proved stable over the 1 hr. stimulation period.

Autoradiographs of cryostat-cut 30μm coronal sections from the same brains reveal highly localized patterns of increased activity-related deoxyglucose consumption (i.e., increased optical density) within a portion of the ipsilateral, but not the contralateral, NTS. Similar increases in autoradiographed NTS optical density are less circumscribed, and predictably less intense, in animals exposed to natural taste stimuli, and they are not found in the control animals. High optical densities in other neural structures (e.g., components of the vestibular and auditory systems) are similar among all animals. These results show that neuroanatomical components of the taste pathways can be directly visualized and partitioned with deoxyglucose autoradiography in correspondence with simultaneously determined electrophysiological response properties of their cells. Further studies will be aimed at more refined electrophysiological and anatomical analyses of the individual neuroanatomical subdivisions associated with each deoxyglucose circumscribed taste pathway.

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240 TASTE DISCRIMINATION, GENERALIZATION, AND THE GUSTATORY NEOCORTEX. J A. Phillips* and J.J. Braun* (SPON: A S. Schwartz). Arizona State University, Tempe, AZ 85281.

The learned discrimination and generalization capacities of rats lacking gustatory neocortex (N=12) were compared to normal rats (N=12) using similar salts as stimuli. All rats were trained to avoid LiCl (.12 M). After training, normal rats (Group NT) were found to generalize to NaCl solutions (.04 M, .12 M, & .36 M) in proportion to how closely they resembled the LiCl training concentration. Group GN generalized along an absolute continuum of stimulus intensity. In addition, at near-threshold concentrations of NaCl (.004 M, .012 M, & .036 M) Group GN did not generalize, whereas Group NT did. Both groups were able to discriminate .12 M LiCl and .12 M NaCl, but Group GN continued to drink more LiCl than Group NT throughout training, suggesting that they may use post-ingestional effects to make such discriminations instead of taste differences.

In Experiment II, 23 rats were trained to avoid LiCl (.12 M). Subsequent aversion generalization tests with NaCl solutions (.12 M, .18 M, & .24 M) supported the results of Experiment I. When the concentration of LiCl was lowered to .06 M, Group NT learned to avoid the solution but Group GN did not. Taken together, these results support the hypothesis that rats lacking gustatory neocortex display elevated association thresholds for detectable taste stimuli.

241 THE ROLE OF NUCLEUS BASALIS IN OLFACTORY BULB RESPONSES TO ODORS IN RABBITS. Harry Potter* (SPON: S.L. Chorover). Dept. of Psych., MIT, Cambridge, Mass. 02139.

The nucleus basalis (NB) includes a group of large, multipolar cells in the basal forebrain which project to the olfactory bulb (OB) and anterior olfactory nucleus (AON). We have examined the activity and responses of these cells while simultaneously recording from units in the OB of awake, partially restrained rabbits. The motivational and arousal state of each rabbit was manipulated by food-deprivation (hunger), sleep, visual and auditory stimulation (arousal), and immobilization (animal hypnosis), as well as by administration of insulin following eating (induced hunger). Records of unit activity from OB and NB were compared with cortical EEG, and nasal air flow recordings obtained simultaneously. While NB units were more sensitive to behavioral state, both NB and OB units responded to odors. Both NB and OB responses to food odors were selectively enhanced during food deprivation and induced hunger, while hypnosis reduced responsiveness to all odors. Insulin induced hunger was also often accompanied by changes in valence of OB or NB unit responses to odor. None of the observed state-dependent odor responses could be accounted for by changes in sniffing patterns. Effects of state manipulation and reversible inactivation of NB (via Lidocaine infusion) upon OB unit responses to odor are examined.

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242 TASTE RESPONSES OF GLOSSOPHARYNGEAL NEURONS OF THE MUD PUPPY (NECTURUS MACULOSUS). David W. Samanen*and Rudy A. Bernard (SPON: P.P.C. Graziadei). Dept. Physiol., Michigan State University, East Lansing, MI 48823.

The electrophysiological responses to four taste stimuli (HCl, NaCl, quinine hydrochloride, and sucrose) were recorded from glossopharyngeal single-fiber preparations of an aquatic amphibian, the mud puppy, Necturus maculosus. The effects of stimulus concentration upon the response magnitude, the response latency, and the form of the response were observed over a 3.5 Log_{10} range. The response was observed to take one of three forms: increasing activity on stimulation, decreasing activity on stimulation, or increasing activity to the distilled water rinse. All response parameters (magnitude, latency, and form) were found in 9 specific combinations (e.g., 1. responses of increasing magnitude and decreasing latency as stimulus concentration increased, or 2. responses of constant magnitude and latency over the range of concentrations.

Following the standard extracellular, macroelectrode recording, the isolation of each neuron was evaluated by computer analysis of the diphasic action potential amplitude. An enumerator computer program, employing the neuron's diphasic amplitude window, counted impulses over the 20 sec test period and pre- and post-stimulus distilled water rinses. To determine the response latency, a fluid switch circuit was used to determine the stimulus delivery time after each experiment.

Most neurons were found to be multiply sensitive (to two or more of the stimuli). However, the neurons also exhibited a degree of chemospecificity, often giving one of the 9 types of responses to one of the 4 stimuli. The type of taste response with constant magnitude and latency over the range of concentrations was stimulus-specific. In contrast, 12 of 24 fibers responded with increasing magnitude and decreasing latency to more than one of the stimuli.

243 CONTRIBUTION OF THE ANION TO THE SALTY TASTE. Susan S. Schiffman* Dept. Psychiatry, Duke Univ., Durham, N.C. 27710.

A group of sodium salts listed on the federal GRAS lists (sodium acetate; sodium ascorbate; sodium bicarbonate; sodium carbonate; sodium chloride; sodium citrate; monosodium glutamate; sodium phosphate, dibasic; sodium phosphate, monobasic; sodium tartrate; sodium succinate; sodium bromide; sodium sulfate) were compared at .2M Na^+ to determine the similarity of their tastes. There were a total of 19 different solutions, with 6 of the salts prepared twice: 1) at the pH normally found at .2M Na^+ and 2) at pH 7.0 adjusted by adding the corresponding acid or CO_2. ALSCAL, a multidimensional scaling procedure developed by Takane, Young, and DeLeeuw (Psychometrika, in press), was applied to the similarity measures to arrange the salts in a multidimensional space with salts tasting similar clustered near one another. The anion had an important contribution, inhibiting the salty taste in some cases and adding additional tastes of its own. Differences between PTC tasters and nontasters were found.

244 LAMINAR DISTRIBUTIONS OF SHORT AXON CELLS IN THE HAMSTER OLFACTORY BULB. S.Schneider*, F.Macrides, B.Davis and W.Youngs*. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The laminar distributions of short axon (SA) interneurons in the main olfactory bulb (MOB) of adult hamsters were studied with rapid Golgi techniques. Six clearly identifiable classes of SA cells were observed and characterized according to somal location, dendritic arborization and axonal ramification. Two of these classes, the Golgi and Blanes cells, had their somata restricted to the interior regions of the MOB and were not observed more superficially than the mitral body layer (MBL). The Blanes cells could be found throughout the granule cell layer (GRL), whereas Golgi cells tended to lie in the superficial GRL and internal plexiform layer (IPL). The dendrites of these two classes extended in all directions from the somata and were not oriented with respect to the layers of the MOB. Their dendrites and axons tended to be restricted to the GRL but occasionally could be traced as far peripherally as the deeper portion of the external plexiform layer (EPL). Two other classes had somata which were restricted to the IPL and MBL, and dendrites which exhibited clear orientations with respect to these layers. One class (horizontal cells of Cajal) had dendrites which ran tangentially in the IPL and MBL, and extended only short distances into adjacent layers. The other class (vertical cells of Cajal) had radially oriented dendrites which extended peripherally into the superficial region of the EPL and centrally for greater distances into the GRL. Both classes gave rise to axons which projected peripherally as far as the border between the EPL and the glomerular layer (GL), but terminated predominantly in the EPL. Another class of SA cells had somata located predominantly in the superficial EPL and to some extent in the periglomerular regions of the GL. They had dendritic and axonal processes which extended far into the GL and appeared to branch around and between individual glomeruli, as well as in the superficial EPL. The sixth class, periglomerular (PG) cells, had somata located throughout the GL. Most PG cells gave rise to one dendritic trunk that arborized within a single glomerulus. Occasionally a second, less elaborate dendritic process emerged from the main trunk or from the soma and extended into an adjacent glomerulus. The axons of PG cells were only partially stained in our material. Such axons were restricted to the GL, but terminal arborizations were not found. These laminar distributions based on morphological observations correlate with electrophysiological data from our laboratory that reveal laminar distributions of response properties for spike generating interneurons in the MOB. (Supported by NSF grant BNS75-07652 and NINCDS grant NS12344)

245 INTENSITY CODING IN THE PONTINE TASTE AREA. Thomas R. Scott and Richard S. Perrotto*. Dep't. Psychol. and Inst. for Neuroscience and Behavior, Univ. of Delaware, Newark, DE 19711.

Increases in taste stimulus concentration have been shown to be represented by monotonically rising response rates from neural populations in the chorda tympani and solitary nucleus. Individual neurons within these populations nearly always show a similar direct relationship between stimulus intensity and discharge rate. In contrast, thalamic taste cells respond idiosyncratically and unpredictably to concentration changes, and population intensity-response functions show occasional non-monotonicity. Third-order neurons of the pontine taste area mediate between solitary nucleus and thalamic cells, and their responses to changing taste intensities were the subject of this study.

Forty single neurons from the pontine taste areas of acute Nembutalized rats were isolated by tungsten microelectrodes. Four qualities representing the four "basic tastes" were used, each at five concentrations covering a range of at least two log units. Pontine taste neurons responded similarly to, but not quite as vigorously as those of the solitary nucleus. NaCl evoked the most robust activity, followed closely by HCl. Sucrose and QHCl were only 35% as effective in stimulating these units. Responses were nearly always excitatory and of short latency. All stimuli elicited monotonic intensity-response functions from the pontine population sample, and all were adequately fitted to power functions with exponents of .18 to .22. Across-fiber patterns of activity within any quality correlated very well across intensities, indicating a high degree of taste constancy. In all of these characteristics, pontine units mirrored the activity patterns of first- and second-order neurons and were discrepant from those of fourth-order thalamic cells.

246 PRIMARY OLFACTORY NERVE AND UNDERWATER ELECTRO-OLFACTOGRAM (EOG) RESPONSES IN THE FRESHWATER EEL. Wayne L. Silver and Don Tucker. Dept. of Biological Science, Florida State University, Tallahassee, Florida, 32306.

Averaged neural activity recorded from small bundles of primary olfactory nerve with Pt-Ir wire electrodes and the "underwater" EOG recorded with calomel electrodes via Ringer-agar filled capillary pipettes were obtained from the freshwater eel. Both response magnitudes increased exponentially with logarithmic increase in stimulus concentration ranging over the 6 log units tested (10^{-8}M to 10^{-2}M), signifying power functions, $R=kC^n$, or exponential functions in terms of log C, $R=(10)^{n\log C}$. However, the exponent n, determined by the slope of a regression line fitted to the points of a log-log response concentration plot, was twice as large for the EOG (.21±.04, N=11) as it was for the neural response (.10±.02, N=8) with amino acid stimuli. Therefore, with decreasing concentrations, the EOG response declines at a rate twice as great as the neural response, suggesting that at lower stimulus concentrations the EOG response may appear to be absent. This may account for the EOG threshold ($10^{-8.2\pm1.1}$M, N=11) being higher than the neural response threshold ($10^{-9.9\pm2.4}$M, N=8). A given electrophysiological threshold concentration was determined from the extrapolation of the regression line to the control level. The control response has been concluded to be due primarily to chemical contaminants.

The freshwater eel has been reported to possess one of the most sensitive olfactory systems of all vertebrates (Teichmann, H., Z. Vergl. Physiol., 42:206, 1959). With behavioral conditioning techniques, threshold concentrations as low as 10^{-18}M were observed for such substances as phenethyl alcohol. Preliminary results in the present study show thresholds for phenethyl alcohol of $10^{-7.5}$M for the EOG and $10^{-9.9}$M for the neural response. Exponents were .15 for the EOG and .09 for the neural response. Although not as low as those obtained with behavioral conditioning methods, the electrophysiological threshold concentrations reported here for the freshwater eel are equal to or lower than those determined in a similar manner for other fish. (Supported by NIH grants, NS-08814 and NS-05258).

247 EFFECTS OF DEAFFERENTATION OF ACCESSORY AND/OR MAIN OLFACTORY SYSTEMS ON INFANT AND ADULT FEEDING BEHAVIOR IN RATS. Pauline J. Singh, Scott Manaker*, and Myron A. Hofer. Dept. Psychiatry, Albert Einstein Col. of Med., Montefiore Hosp., Bronx, N.Y. 10467.

It has been reported that 18 to 19-day-old pups which have undergone intranasal perfusion with 5% $ZnSO_4$ solution have exhibited deficiencies in suckling but not in solid food ingestion (Alberts, 1976). This indicates that the accessory system (which is not deafferented by $ZnSO_4$ treatment) is involved in solid food ingestion but relatively unimportant in normal nursing behavior. In this experiment the role of the accessory system as well as that of the main system in infant and adult feeding were more extensively investigated. Seven litters were observed, each litter having been culled to 7 pups. At 18 to 19 days of age, pups within each litter underwent vomeronasal nerve section (VN), which results in deafferentation of the accessory system; intranasal perfusion with 5% $ZnSO_4$ solution (Zn); bilateral olfactory bulbectomy (BOB), which results in deafferentation of main and accessory systems; or surgical control procedure (SC). Some pups were left intact as normal controls (N). After 24 hrs with their lactating mother, Zn and BOB pups lost weight, whereas VN, SC, and N pups gained weight (differences at $p<.01$). Pups were observed for latency to approach and ingest food pellets after 24 hrs of deprivation from their mother and solid food. Although their level of general activity was not different from that of other groups, BOB pups had a significantly longer latency ($p<.05$) to food pellet ingestion than VN, SC, or N pups; whereas the latency of the Zn pups fell between those of the VN, SC, or N pups on one hand and that of the BOB pups on the other and was not significantly different from any. Results of VN pups indicate that an intact accessory system is not necessary for suckling or solid food feeding. Deafferentation of the main system (Zn pups) is followed by a definite suckling deficiency; and BOB, which includes deafferentation of both systems, results in marked deficiencies in both types of feeding.

248 RAT CHORDA TYMPANI RESPONSE TO NaCl: ADAPTATION OR TRANSFORMATION? David V. Smith and Susan Plock*. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Both the transient portion of the integrated chorda tympani response and the initial burst of impulses in single peripheral neurons are extremely sensitive to the rate of stimulus change (Smith & Bealer, 1975). In addition to this rate-sensitive transient, the rat chorda tympani response to NaCl shows a slow exponential decay during the "steady-state" phase of the response (Smith, Steadman, & Rhodine, 1975). Following adaptation to NaCl, a period of postexcitatory depression occurs in which there is suppression of both the transient and "steady-state" components of the response to a test stimulus. The magnitude of this postexcitatory depression is dependent upon the concentration (Smith & Bealer, 1976) and duration (Smith, Bealer, & VanBuskirk, 1977) of the adapting stimulus; the time course of the recovery of excitability during a subsequent distilled water rinse mimics the time course of the slow decline in the response to the adapting stimulus. Responses of taste receptor cells do not show a transient component (Balnave, 1976; Sato, 1976), which suggests that the response is proportional to the number of occupied receptor sites rather than to their rate of binding (see Heck & Erickson, 1973). However, the rise time of the receptor potential has been shown to be sensitive to the rate of stimulus flow (Sato, 1976) and the slow adaptive process reflected in the decline in the chorda tympani response to NaCl is also seen to occur in the taste receptor cell (Ozeki, 1971). These data suggest a model of the probable sequence of events leading to the first-order neural response to NaCl. The taste receptor potential is proposed to be proportional to the number of occupied receptor sites minus the magnitude of a slowly increasing adaptive process. Time course analysis of the response of the rat chorda tympani nerve to HCl shows that the slow decline seen in the response to NaCl does not occur to all compounds, suggesting that this adaptation is the property of a specific receptor mechanism. This adaptive process to NaCl dissipates only slowly during the rinse period, producing the prolonged period of postexcitatory depression. The response of the chorda tympani nerve is proposed to be sensitive to the amplitude of the receptor potential and to its rate of change, i.e., the first-order nerve adds a differential to the taste receptor input. The decline from the initial transient reflects the transfer function between receptor cell and first-order nerve, while the slow decline represents an adaptive mechanism. Thus, the rate sensitivity of the chorda tympani nerve can be accounted for under an occupation model (Beidler, 1954) of taste receptor stimulation.

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249 NEONATAL 6-HYDROXYDOPAMINE ALTERS SPECIFIC OLFACTORY PREFERENCES AND BRAIN CATECHOLAMINE CONTENT IN RATS. S.K. Sobrian, E.M. Marasco* and C. Cornwell-Jones*. Dept. Psych., Princeton Univ., Princeton, NJ 08540.

Preferences of Sprague-Dawley rat pups for conspecific odors and catecholamine(CA) content of olfactory brain regions were altered by neonatal 6-hydroxydopamine(6-OHDA) treatment. Responses to arbitrary (non-ethological) odors, whether familiar or novel, were not altered. Littermates reared in cedar shavings were injected subcutaneously on Days 0-3 with 50 ug/g of 6-OHDA or 0.9% saline. Pups were tested in a 2-choice situation requiring a locomotor response. On Days 8-14, saline-injected pups spent significantly more than 50% of test time over cedar nest shavings in favor of natural cedar, while 6-OHDA pups were indifferent to either odor. Group differences were significant on Days 10-14. This 6-OHDA induced change was specific to social odor. It did not reflect a deficit in locomotor ability: pine-reared 6-OHDA and saline pups 5-14 days of age avoided cedar odor, spending significantly less than 50% of test time over natural cedar in preference to natural pine. Therefore, drug treatment did not impair ability to orient to odors. Moreover, a general deficit in olfactory learning can not account for these results. Both 6-OHDA and saline pups reared in cedar acquired a preference for this normally aversive odor. Drug-induced behavioral deficits coincided with reductions in CA content of several olfactory innervated brain regions. Norepinephrine in olfactory bulb and prepiriform cortex was significantly lower than control levels at Day 14 (66.8% and 63.4%, respectively). Dopamine in olfactory tubercle was unaltered; amine changes in amygdala did not parallel behavioral changes.

Drug treatment also altered the preference of pine-reared pups for conspecific but not arbitrary odors. Pups injected Days 4-7 were less attracted to pine nest shavings than saline controls; aversion to lemon odor was unaltered. Injections beginning after postnatal week one (Days 8-11) had no effect on the development of odor-guided behavior.

Therefore, 6-OHDA produces a selective deficit in the development of conspecific but not arbitrary odor preference. This deficit is independent of rearing conditions, does not reflect motoric or general olfactory learning impairment, but coincides with CA alterations in brain regions receiving olfactory innervation.

250 2-DEOXYGLUCOSE UPTAKE PATTERNS IN RAT OLFACTORY BULB UNDER DIFFERENT ODOR CONDITIONS. William B. Stewart*, John S. Kauer and Gordon M. Shepherd. Dept. of Physiology and Section of Neurosurgery, Yale Univ. Sch. of Med., New Haven, Conn. 06510.

Previous work with the 2-deoxyglucose (2DG) method has revealed localized regions of glucose uptake in the rat olfactory bulb (Sharp, Kauer & Shepherd, Brain Res. 98:596-600, 1975). The glomerular layer, where the olfactory axons terminate, is especially active in this regard. Preliminary experiments suggest that spatial variations in the density of this layer are associated with specific odor stimulation, both in rats (Sharp, Kauer & Shepherd, 1975; Sharp, Kauer & Shepherd, J. Neurophysiol. (in press) and in tree shrews (Skeen, Brain Res. 124:147-153, 1977). We have made a systematic study of these patterns under a variety of carefully controlled stimulus conditions. In olfactory bulbs of rats exposed to purified air, localized 2DG uptake was absent or minimal. Animals breathing room air had small distinct foci, as previously reported (Sharp, Kauer & Shepherd, 1975). These were predominately located in the midlateral and posteromedial portions of the bulb. Animals exposed to the odor of one of the following substances: dimethyl disulfide (a putative pheromone; Singer et al, Science 191: 948-950, 1976), amyl acetate and camphor, showed patterns which were relatively distinct from each other. The effects of different odor concentrations on the patterns have also been studied. The significance of the patterns for sensory processing will be discussed. Certain behavioral conditions that may contribute to the variability in the patterns will be identified.

251 AMNIOTIC FLUID CONTROL OVER THE FIRST SUCKLING RESPONSE OF NEWBORN ALBINO RATS. Martin H. Teicher* and Elliott M. Blass. Dept. Psychology, Johns Hopkins University, Baltimore Md. 21218.

Olfactory control over neonatal rats first suckling experience was studied within 2 hr. postpartum. Pups were hand-held and slowly brought into contact with a teat of their anesthetized dam. 180 pups were partitioned into the following groups: control; washed nipple (3:2 solution of methylene chloride and 95% ethanol) and washed nipple coated with one of the following: amniotic fluid, parturient mother saliva, virgin female saliva, parturient mother urine, amyl acetate, vanilla extract, or isotonic saline. A rat was tested in only one condition. Pups readily attached to the unwashed (control) mother. Nipple wash essentially eliminated attachment. This strongly suggests that a substance either secreted or placed by the mother on her mammary area is necessary to elicit suckling in her newborn. The only substances which returned suckling to control levels were amniotic fluid and parturient mother saliva. None of the other fluids raised attachment above wash levels. This suggests chemical specificity. These data also suggest that neonatal rats possess and utilize olfactory sensitivity at birth.

252 GUSTATORY-RESPONSIVE NEURONS IN THE HAMSTER PARABRACHIAL PONS. Richard L. Van Buskirk and David V. Smith. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

The parabrachial pontine region contains a significant relay for gustatory responses in both the rat (Norgren & Leonard, 1971; Norgren & Pfaffmann, 1975; Perrotto & Scott, 1976) and the cat (Bernard & Nord, 1971), suggesting the potential generality of such a relay for mammals. The present study shows that taste responses are also found in the pontine parabrachial region of the hamster. In the cat, gustatory responsiveness is confined to the area ventral to the brachium conjunctivum (Bernard & Nord, 1971), but in the hamster gustatory responses are found both dorsal and ventral to the brachium conjunctivum, as is the case in the rat (Norgren & Pfaffmann, 1975). However, all stimulation in the hamster has so far been confined to the anterior portion of the tongue. Anesthesia, general surgical procedures, and stimulus control were comparable to earlier work on the hamster NTS (Travers & Smith, 1976). With the head tilted forward at 27^{o} off the horizontal plane, the parabrachial area is accessible below the suture of the occipital and interparietal plates, and involvement with the transverse sinus is thereby avoided. In this stereotaxic orientation, gustatory responses were found between 3.9 and 4.5 mm rostral to obex, between 3.6 and 4.2 mm below the cerebellar surface, and centered along a line 1.35 mm from the midline. Gustatory stimulation included the four classical stimuli (0.03 M NaCl, 0.003 M HCl, 0.1 M sucrose, and 0.001 M QHCl) plus an array of 14 other compounds. Most of these pontine taste neurons could be characterized as "multiple-stimulus" neurons rather than "best-stimulus" neurons, even under the most stringent criteria (Pfaffmann et al., 1976), and were found to respond to a wide variety of stimuli. In fact, statistically significant responses to at least three of the four classical gustatory stimuli have been found in most of the neurons examined to date. Neither spontaneous rate nor the range of firing rates to gustatory stimuli appears different from that in hamster NTS (Travers & Smith, 1976). In several cases, when the animal was breathing on its own, neurons firing in synchrony with either inhalation or exhalation were found at the same medial-lateral, dorsal-ventral location as gustatory-responsive neurons located just rostral and caudal to these respiratory units. When the animal was mechanically respired there were no respiratory-synchronous units found in this area, but neurons with highly regular spontaneous firing rates were quite commonly found interpolated among small groups of taste-responsive neurons.

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253 OBSERVATIONS ON THE NORMAL STRUCTURE OF THE VOMERONASAL APPARATUS OF GARTER SNAKES. Ruu Tong Wang*, John L. Kubie* and Mimi Halpern. Dept. of Anatomy and Cell Biology and Program in Biological Psychology, Downstate Medical Center, Brooklyn, New York, 11203.

The organization of the vomeronasal mucosa of garter snakes contrasts sharply with the organization of the olfactory mucosa. The vomeronasal apparatus consists of a superficially positioned epithelium, a very thick intermediately located bipolar(Bp) cell layer and a deeply situated layer of undifferentiated(Ud) cells. The epithelium is separated from the Bp cell layer by a basement membrane, connective tissue and blood vessels.

The epithelium contains a single layer of columnar cells with apical microvilli and oval nuclei situated basally. The dendrites of Bp cells lace their way between adjacent columnar cells as they pass toward the luminal surface.

The Bp cell layer is 20 to 30 cell bodies deep and organized into hexagonal columns oriented perpendicular to the luminal surface. Pigmented connective tissue septi containing blood vessels separate adjacent columns. Within each column dendrites and axons aggregate into bundles which form parallel arrays also oriented perpendicular to the luminal surface.

The layer of Ud cells is 6 to 10 cells deep and is organized into the same hexagonal columns as the Bp cell layer. Within each column several mitotic figures can be seen among the Ud cells.

Electron microscopic examination of the epithelium reveals that membrane specializations exist between adjacent columnar cells and between the apices of columnar cells and Bp cell dendrites at the luminal surface. Bipolar cell dendrites traveling through the epithelium contain large numbers of neurofilaments and elongated mitochondria while columnar cells contain polyribosomes, secretory or lysosomal granules and a few large mitochondria, but no neurofilaments. The round nuclei of Bp cells are surrounded by substantial perikarya containing large amounts of concentrically oriented, tightly packed smooth endoplasmic reticulum (ER) and rough ER in the polar regions of the cell body. Those Bp perikarya closer to the epithelium contain more smooth ER, a greater number of lysosomes and larger lysosomes than Bp perikarya close to the layer of undifferentiated cells.

Undifferentiated cells are larger than Bp cells, have very large oval to irregularly shaped nuclei and scanty perikaryal cytoplasm. The predominant cytoplasmic organelles of Ud cells are polyribosomes. Very small quantities of rough ER and few mitochondria are observed in them.

The axons of Bp neurons traverse the layer of Ud cells and collect between that layer and the bony capsule to form the bundles of the vomeronasal nerve. Satellite cells ensheath the axons forming bundles of hundreds of axons each.

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254 SOME RESPONSE PROPERTIES OF SINGLE UNITS IN THE OUTER LAYERS OF THE HAMSTER OLFACTORY BULB. W.Youngs*, F.Macrides, S.Schneider* and B.Davis (SPON: R.Hall). The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Extracellular recordings from single units in the mitral body (MBL), external plexiform (EPL) and glomerular (GL) layers of the hamster olfactory bulb (OB) reveal three major categories of neurons which can be distinguished according to their pattern of responding to electrical stimulation of the ipsilateral olfactory mucosa (OM) and lateral olfactory tract (LOT). Neurons belonging to the first category responded with a single, fixed latency spike to single shocks of the LOT and were classified as output neurons. Approximately half of them were excited by stimulation to the OM, although a higher percentage of the output neurons with long versus short LOT latencies were excited by OM stimulation. This differential sensitivity to electrical stimulation of the OM may reflect a functional dichotomy of output neurons: smaller cells more sensitive to changes in overall level of OM activation, larger cells more sensitive to specific spatiotemporal patterns of activation. Interneurons composing a second category were recorded in the GL and were localized at the boundaries of glomeruli by iontophoresis of dye at the recording sites. These interneurons often displayed a high frequency, "bursty" firing pattern and showed either no clear response or suppression of spontaneous firing following single and multiple shocks to the LOT. Many of the GL interneurons were excited by OM stimulation. As a group they had significantly shorter OM latencies than output neurons, suggesting that they receive direct excitatory inputs from the OM. Interneurons located predominantly in the EPL compose a third category of neuron. They had low spontaneous firing rates and tended to be excited by OM stimulation with longer latencies than output neurons and GL interneurons. They also tended to be excited by LOT stimulation, showing variable latency bursts related to stimulus strength. Their OM and LOT latencies were consistent with an intrabulbar excitatory pathway to them through output neuron collaterals. Most of the EPL interneurons were suppressed by electrical stimulation of the contralateral OB. Since such stimulation is known to be excitatory to granule cells it is likely that EPL interneurons are short axon (SA) cells. The possibility that interneurons recorded in the GL are granule cells also may be ruled out since granule cell processes do not extend into the GL and do not receive OM inputs. We postulate that they are periglomerular (PG) cells, which are thought to be inhibitory to output neurons. The possibility that SA cells of the EPL are inhibitory to PG cells, and thus provide a disinhibitory pathway for output neurons, will be discussed. (Supported by NSF grant BNS75-07652 and NINCDS grant NS12344)

COMPARATIVE NEUROBIOLOGY

255 TELENCEPHALIC VISUAL RESPONSES IN THE PAINTED TURTLE, CHRYSEMYS PICTA. A. H. Bass, M. L. Andry* and R. G. Northcutt. Div. Biol. Sci's., University of Michigan, Ann Arbor, MI 48109.

Recent anatomical investigations demonstrate a widespread distribution of primary retinal targets in the mesencephalon and diencephalon of turtles (Bass and Northcutt, 1975; Bass, 1976). Electrophysiological methods were used to investigate the possibility that such a multiplicity of retinal targets reflects a plurality of visual regions in the telencephalon.

Eleven painted turtles, Chrysemys picta, were analyzed for multiple unit activity responsive to photic stimulation. Animals were anesthetized with Nembutal (25 mg/kg, i.p.); multiple unit activity was recorded with platinum-irridium electrodes (75 u). Superficial units were associated with surface negative evoked responses; deeper units were associated with response component polarity inversions. On and/or off cluster activity occurred only to contralateral stimulation.

Surface recorded evoked responses were comparable to those reported by Belekhova et al. (1964) and by Karamian et al. (1966). Histological examination of electrode tracks revealed sites of multiple unit activity in the following regions: pars dorsomedialis and dorsolateralis of dorsal cortex; dorsomedial segment of posterior dorsal ventricular ridge; and a dorsolateral segment of anterior dorsal ventricular ridge which appears to correspond to the rotundal target of Hall and Ebner (1970) in Pseudemys.

This research was supported by NIH Fellowship 1 F22 NS 02622 and NIH Grant 2 R01 NS 11006.

256 ANATOMICAL STUDIES ON AFFERENT AND EFFERENT CONNECTIONS OF THE MORMYRID ACOUSTICOLATERAL AREA. C.C.Bell and C.J.Russell, Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon 97210.

There are three types of electroceptors in mormyrids; ampullary,mormyromast, and Knollenorgan. Electrophysiological and anatomical work has been done on afferent projections from these receptors to the CNS. The present study extends these findings.

Electroreceptor afferents from dorsal posterior skin run in the dorsal branch of the posterior lateral line nerve. Those from ventral posterior skin run in the ventral branch. The latter branch also contains ordinary (mechanoreceptor) lateral line afferents and efferents. We labelled axons of each branch to determine termination sites. Under anesthesia, a branch was exposed and cut near the sensory ganglion. HRP in a weak solution of DMSO (dimethyl sulfoxide) was put on the cut end. After closing the wound, animals survived 5-7 days. Sections were prepared in the usual way. DMSO enhances diffuse, agranular filling with HRP (Keefer et al, Neuroscience Letters, 3 (1976) 233-237). Filled axons could be seen in nerve and CNS.

There are two structural discontinuities on each side of the cortex of the mormyrid posterior lateral line lobe (PLLL). These run longitudinally,defining three zones; medial, dorsolateral, and ventrolateral. Following dorsal branch labelling filled terminals are seen in three separate patches, medially within the medial zone and ventrally in each lateral zone. With ventral branch labelling terminals are lateral in the medial zone and dorsal within the lateral zones. The results indicate three distinct mappings of electroreceptor afferents onto PLLL cortex. Electrophysiological studies have shown that the ventrolateral zone receives ampullary while the other two zones receive mormyromast input.The somatopy seen here confirms that seen physiologically in the ampullary and medial mormyromast zones. Afferents also end in the nucleus of PLLL. These are probably from Knollenorgan receptors. Finally, terminals are found in the anterior nucleus just deep to the crista cerebellaris following ventral but not dorsal branch filling, indicating that mechanoreceptors end here, as has been suggested by others.

Injection of HRP (in distilled water) into ganglion lateralis of the mesencephalon shows heavy projections from all three zones of the contralateral PLLL. Both ganglion and beard cells project. Neurons of the nucleus of PLLL do not go to g. lateralis but to g. exterolateralis.

The results indicate that mormyromasts are more similar to ampullary than to Knollenorgan receptors in their central connections. (Supported by NIH (NINCDS-06728) and NSF (BMS73 - 06867))

257 TELENCEPHALIC AFFERENTS AND SDH DISTRIBUTION IN POLYPTERUS. M. R. Braford, Jr. and R. G. Northcutt. Div. Biol. Sci's., University of Michigan, Ann Arbor, Michigan 48109.

The distribution of succinate dehydrogenase (SDH) in the telencephalon of the bony fish Polypterus palmas was studied with the method of Pearse. High concentrations of SDH were found in the glomerular layer of the olfactory bulb and in the superficial neuropil of all pallial areas. The pallial distribution occurs in a band of varying thickness which occupies the superficial one-third of the neuropil of Pl, extends almost to the ependymal surface in a restricted rostral part of P2, and is narrower in the remainder of P2 and in P3. Following lesions of the olfactory bulb, terminal degeneration was seen in the superficial one-third of Pl. Large diencephalic lesions produced terminal degeneration in P2 and P3. The combined distribution of the olfactory and ascending projections coincides in precise detail with the high SDH concentrations in the pallium. Areas of moderate or low SDH concentrations in the subpallium also correspond closely to the areas which receive olfactory or ascending inputs. Similar correlations have been reported for sensory areas in the telencephalon of reptiles and mammals. Although the nature of the ascending projections in Polypterus remains to be determined, these data suggest that SDH distributions are reliable markers for sensory projection areas and may be useful as predictors in the absence of experimentally determined connections. (Supported by NSF Grant GB-40134 and NIH Grant 2 R01 NS 11006.)

258 RETINOTHALAMIC PROJECTIONS IN LIZARDS AS REVEALED BY ANTEROGRADE AUTORADIOGRAPHY. Ann B. Butler and R. Glenn Northcutt. Dept. Anat., Georgetown U., Wash., D.C. 20007 and Div. Biol., U. Mich., Ann Arbor, Mi. 48104.

While the retina has been found to project to multiple sites in the dorsal thalamus of birds, most previous studies in reptiles have identified only one dorsal thalamic retinal target, nucleus geniculatus lateralis pars dorsalis (PD). A reexamination of the retinal projections in two lizards, Iguana iguana and Gekko gecko, was therefore undertaken. Five animals received intraocular injections of (^{3}H)proline, 9.4 μC/μl. The brains were fixed by perfusion 1-6 days postoperatively. Mounted paraffin sections were coated with NTB3 emulsion and developed after 20 days exposure.

In the contralateral thalamus a number of retinal targets could be identified in both species. The pars ventralis (PV) and pars dorsalis (PD) of the geniculate and nucleus ventrolateralis pars ventralis (VLv) were found to receive retinal projections as previously described (Butler and Northcutt, '71, Brain Res., 26: 1-13; Northcutt and Butler, '74, JCN, 157: 453-466). Additionally, the retina projects to (1) a nucleus lying dorsal to PD, the dorsal optic nucleus (DON), (2) a nucleus which caps the dorsal surface of nucleus rotundus, nucleus dorsocentralis (DC), (3) a nucleus lying between PD and nucleus dorsolateralis anterior, nucleus intercalatus thalami (IC), and (4) part of nucleus ventrolateralis pars dorsalis (VLd). Part of DON was previously included as the dorsal portion of PD in Iguana and degeneration was also previously charted in this region in Gekko. Ipsilateral retinal projections were traced to the more laterally lying of these nuclei: PV, PD and DON. If more than one of these thalamic visual nuclei is found to project to the telencephalon, the relationship of the PD of reptiles to the dorsal lateral geniculate nucleus of mammals will have to be re-evaluated. (Supported by NIH Grant No. NS11006 and NSF Grant No. GB-40134 to RGN and NIH Grant No. NS12966 to ABB.)

259 THE ACCESSORY OPTIC SYSTEM IN TELEOST FISHES. T.E. Finger and H.J. Karten, Dept. Anat. & Neurobiol., Sch. Med., Washington Univ., St. Louis, Mo. 63110 and Dept. Psychiatry, State Univ. N.Y. at Stony Brook, Stony Brook, N.Y. 11794.

Karten, et al (PNAS 1977) have demonstrated that the accessory optic nuclei (AON) in pigeon are cell groups which both receive a direct retinal input and project directly to end as mossy fibers in caudal cerebellum. A homologous AON system in other vertebrates should be expected to have similar connections. In order to determine if piscine forms possess a cell group which might meet these criteria, HRP was injected into the corpus cerebelli of catfish and goldfish in order to identify cells projecting to cerebellum. In other specimens, the eye was injected with tritiated proline and the brain processed for autoradiography in order to demonstrate retinal projections.

In both species, we were able to identify two distinct nuclei which both received retinal input and projected to cerebellum. These cell groups lie along the medial and ventral aspect of the rostral tectum. The more ventral group in goldfish forms a spherical nucleus roughly 200 μ in diameter and lies ventrolateral of Schnitzlein's n. Rotundus and immediately dorsal to the optic tract as it courses into the ventral tectum. The dorsal AON in goldfish lies along the junction of tegmentum and tectum at the level of the posterior commissure. Cells in this dorsal nucleus lie within the boundaries of what has been termed area pretectalis by Schnitzlein. In catfish, these AON are connected by a band of cells, whereas in goldfish, the two groups are separated.

Karten et al found that the avian AON receive input from only the displaced ganglion cells. The possibility that specific retinal targets may receive input from only a specific class of ganglion cells in teleosts must also be considered.

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260 COMPARATIVE ASPECTS OF NICOTINIC AND MUSCARINIC ACETYLCHOLINE RECEPTORS IN BRAIN, by Renee Foders and P.M. Salvaterra, Div. of Neurosciences, City of Hope National Med Ctr., Duarte, CA. 91010.

Nicotinic and muscarinic acetylcholine receptors were studied in crude brain membrane fractions by ^{125}I-αbungarotoxin (^{125}I-αBTX, McQuarrie et al., J. Biol Chem 251, 6327, 1976) and ^{3}H-quinuclidinyl benzilate (^{3}H-QNB, Yamamura & Snyder, Proc. Natl. Acad. Sci., 71, 1725, 1974) binding. All species studied (rat, rabbit, mouse, goldfish, frog & Torpedo) showed similar receptor levels in whole brain and exhibited pronounced regional differences. In the fish and frog ^{125}I-αBTX and ^{3}H-QNB binding is highest in optic tectum lower in forebrain and lowest in cerebellum. In Torpedo ^{125}I-αBTX binding shows cerebellum > optic tectum > olfactory lobe > electromotor nucleus while ^{3}H-QNB shows olfactory lobe > electromotor nucleus > cerebellum > optic tectum. For mammals ^{3}H-QNB binding is caudate > hippocampus > cerebrum > cerebellum while ^{125}I-αBTX is variable with cerebellum usually the lowest. While all regions have higher ^{3}H-QNB binding the ratio of ^{3}H-QNB to ^{125}I-αBTX binding varies 15-400 fold indicating the relative importance of muscarinic vs. nicotinic receptors. This point is illustrated by data from hippocampus and caudate of two mouse strains:

Strain	Hippocampus		Caudate	
	^{125}I-αBTX[a]	^{3}H-QNB[a]	^{125}I-αBTX	^{3}H-QNB
Swiss	.038	1.12	.016	1.55
C58/J	.026	0.62	.003	1.32

[a] pmoles/mg

The Swiss mouse is higher in both markers than the C58/J, however, the ratio of ^{3}H-QNB to ^{125}I-αBTX is about the same in hippocampus but is 4-5 times higher in caudate of the C58/J. Choline acetyltransferase (CAT) levels were determined (Fonnum, Brochem J. 115, 465, 1969) and found in general to parallel ^{3}H-QNB binding, but it is interesting to note a higher CAT acitivity in the hippocampus and caudate of the C58/J relative to the Swiss mouse.

261 GENETIC APPROACHES TO BEHAVIORAL DISORDERS IN MAN. Benson E. Ginsburg. Behav. Genet. Lab., Dept. Biobehavioral Sci., Univ. of Conn., Storrs, CT 06268.

Genetically based behavioral disorders in man include those in which there is strict determinism associated with anomalies definable at the chromosomal, genic, and/or mechanism level; those in which there is a correlation with polymorphic markers, suggesting a genetic susceptibility associated with particular chromosomes or chromosomal regions; and those for which multiple predisposing genetic bases exist, suggested by genealogical studies and twin studies compatible with a genetic diathesis but not with one particular mode of inheritance in association with a given syndrome. The latter are of particular interest because where multiple genetic etiologies are involved, the analysis of causal mechanisms will depend upon a suitable genetic taxonomy deriving from genealogical and marker studies in which the multiple genetic bases within a syndrome can be separately followed. Twin studies and work with genetically uniform strains of mice point to the possibility that individuals with identical encoded genotypes may not have identical effective genotypes; i.e., that different genes may be activated. Findings in our laboratory illustrate the efficacy of this approach using familial dyslexia, hyperkinesis, tendencies to aggression, convulsive seizures, and developmental differences in reaction to alcohol. Each of these have diverse genotypes within the syndrome, and the mechanisms associated with the behaviors are genotype rather than phenotype dependent. (Supported by NIH AA 00297, NIMH MH 28021, and by a grant from The Grant Foundation, Inc.).

262 INFRARED AND VISUAL ORGANIZATION OF THE TECTUM OF BOID SNAKES. Eric Haseltine*, Len Kass and Peter Hartline.(SPON: Delores Schroeder). Dept. of Psych. Indiana Univ., Bloomington,Ind.47401 and Dept. of Physiology and Biophysics, Univ. of Ill., Urbana,Ill.

Single and multiunit recordings were made in the tecta of two species of boid snake, Python reticulatus and Corrallus endyris. These snakes possess at least 16 infrared sensitive labial pits.

The topographic organization of visual input to tectum was similar to that of the infrared projection, but maps of the two modalities were not in precise register. Rostral electrode placements were associated with nasal infrared and visual fields,and lateral penetrations produced receptive fields in both modalities situated inferiorly.

Seventy percent of neurons for which labial pit input could be determined were driven by one pit only, and 30% were driven by two or more pits.

Single unit infrared receptive fields were found to be consistently larger than visual fields. Visual fields in P. reticulatus averaged 24° in width,whereas infrared fields averaged51°. Mean visual receptive field width in C. endyris was 14°, while infrared fields were 50° wide one the average.

Bimodal neurons(those responding either to visual or infrared stimuli) were commonly encountered in the tectum of C.endyris. Both visual(average field 34° wide) and infrared(average field 60° wide) receptive fields of these units were larger than those of simple visual and infrared units respectively. Response dynamics of bimodal neurons differed according to the modality of stimulation. Strictly visual stimulation produced a phasic "on" response,followed by and "off" response.Purely infrared stimuli elicited a phasic"on" response with no "off" response. Pronounced post-excitatory depression typically followed response to an infrared stimulus. Temporal characteristics of visual and infrared componants of bimodal neuronal responses, were similar to those of purely visual and infrared units respectively.

263 ACTIVITY-INDUCED HYPERPOLARIZATION IN A MAMMALIAN SENSORY NEURON. Richard A. Jaffe and Sanford R. Sampson. Biol. Dept., Battelle-Northwest, Richland, WA. 99352 and Dept. Physiol., UCSF, San Francisco, CA. 94143.

A prolonged hyperpolarization of the membrane potential following repetitive activity has been demonstrated in several vertebrate and invertebrate neurons. It has been suggested that this hyperpolarization may affect nerve function either by altering the threshold for excitation or by causing conduction block. In a small population of neurons in both cat and rabbit nodose ganglia, repetitive action potentials generated in the cell body caused a persistent hyperpolarization. Measurements were made on nodose ganglia and vagus nerves removed from anesthetized cats or rabbits, maintained in vitro and superfused at 37°C with solutions approximating the ionic composition of extracellular fluid. Intracellular recordings were obtained with glass micropipets filled with 3 M KCl or 5 M KAc. In contrast to post-tetanic hyperpolarization, activity-induced hyperpolarization in nodose ganglion neurons began with the first action potential and persisted no longer than 5 sec after stimulation was stopped. The magnitude of this hyperpolarization was often as high as 10-15 mV and appeared to be dependent on the frequency of discharge, some hyperpolarization being observed at frequencies as low as 1-2 spikes per second. This persistent hyperpolarization occurred only if action potentials were generated in the cell body, and sometimes caused failure of somatic spike generation. The mechanism by which repetitive activity induces hyperpolarization in nodose ganglion neurons is not known. The possibility that it is simply a summation of afterhyperpolarizations seems unlikely, because the duration of the afterhyperpolarization rarely exceeded 30 msec, while activity hyperpolarization was seen with somatic spikes occurring as infrequently as 1-2 per second. It is suggested that the hyperpolarization following repetitive activity in nodose ganglion neurons could, in large part, be accounted for by specific changes in membrane conductance. Further studies will be necessary to confirm this hypothesis and to determine which species of ion are involved.
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264 ANATOMICAL BASIS FOR VISUAL AND NONVISUAL INTEGRATION IN PIT VIPER TECTUM. Leonard Kass, Michael S. Loop and Peter H. Hartline. Dept. Physiol., Univ. of Illinois, Urbana, Il. 61801

The optic tectum of the rattlesnake is used as a model for integration of visual and nonvisual sensory modalities (Hartline, Loop,Kass; these abstracts). In this study we examine the anatomical layering of visual, infrared, and bimodal tectal neurons and their spatiotopic organization.

Visual and infrared cell layers were identified in the tectum of the pit vipers Crotalus viridis and Sistruris melitus. 48 lesions were made after recording visual or infrared activity at varying electrode locations in the tectum. Histological reconstructions of these lesions (Prussian Blue staining technique, Akert and Welker, '61) were correlated with the micrometer depth readings of 271 visual, infrared, and bimodal single unit recordings. We used a thermoneutral black wand against a light background as the visual stimulus and a warm soldering iron in the dark as the infrared stimulus. The visual cell layer extends across the entire tectum from approximately the level of the posterior habenula to the anterior border of the posterior corporo quadrigemina. The visual cell region extends across the midline and comprises zones 7-13, i.e., the superficial 600-700 microns of the optic tectum (stratum fibrosum et griseum superficiale and stratum griseum centrale). The infrared cell group is found everywhere in the tectum below the visual laminae (layers 1-7). Eighty percent of the infrared neurons are found between 500-1200 microns from the surface of the optic tectum. In layer 7 (stratum griseum centrale) the visual and infrared cell groups are mixed; bimodal neurons which respond to a combination of visual and infrared input are located predominately in this layer.

Spatiotopic tectal maps for both modalities were constructed from receptive field centers for superficial (visual) and deep (infrared) recordings with multi-unit electrodes. In both modalities , rostral loci yielded anterior (nasal) fields; medial loci yielded superior fields. Thus, the axes of the spatiotopic maps for both modalities are aligned similiarly across the tectal surface. But the maps differ in detail. Corresponding meridian lines do not have the same shape; the magnification factor (mm tectal locus separation / degree field center displacement) is about 1.5 times greater in the infrared map than is found in the visual map. This requires that the same tectal sublayer receives information (visual and infrared) from two disparate regions of space as do the bimodal neurons.

The approximate similarity of spatial organization of the visual and infrared systems in the optic tectum probably allows orientation behavior to be mediated by either modality.

265 DIFFERENTIAL RESPONSES TO TEA AND BARBITURATES IN FOUR IDENTIFIED LEECH NEURONS: ROLE OF CA. Anna L. Kleinhaus and James W. Prichard. Dept. Neurol., Yale Med. Sch., New Haven, CT. 06510

Previous work from this laboratory showed that tetraethylammonium chloride (TEA) and barbiturates interfered with action potential repolarization in leech Retzius cells. However, the drugs acted by different mechanisms. TEA apparently blocked a K current which normally repolarizes the cell and allowed the effect of a late divalent cation conductance mechanism to dominate membrane behavior during excitation (Kleinhaus and Prichard, J. Physiol. 1975). Barbiturates, on the other hand, interfered with the repolarization mechanism in some other way, possibly by blocking a Ca-activated K conductance in the Retzius cell (Kleinhaus and Prichard, J. Pharm. exp. Ther., (in press).

TEA applied both extracellularly in the bath and intracellularly by iontophoresis prolonged the action potentials of leech sensory neurons. The average value for the increase above control of action potential durations after extracellular application was 500% for N cells, 240% for P cells, and 162% for T cells. With intracellular application the respective increases were 422%, 185%, and 141%. Both amplitude and duration of the TEA-prolonged action potentials were directly related to the Ca concentration. The ability of these neurons to sustain active membrane responses to depolarization when Ca was the only cation available to carry current was found to segregate the cells in the same order. This correspondance suggested that the differential responses to TEA could be explained by variations among the cells in the numbers of Ca conductance channels available to affect membrane behavior when outward K currents were largely blocked.

Pentobarbital and methohexital, 0.1-1.0 mM, also prolonged the action potentials of leech neurons, and in the same order that TEA did. Resting membrane potential and maximum rate of rise of action potentials were also affected, but not in the same order. The effect of Ca on the barbiturate-prolonged action potentials was the opposite of its effect on the TEA-prolonged ones. In all four cells the barbiturate prolongation was reversed by Ca and favored by a reduction in its concentration.

The barbiturates thus interfered with repolarization in all the cells, but the effect was greater in those which appeared to have larger Ca conductances. These observations and our earlier findings on the Retzius cell are compatible with the theory that barbiturates block a Ca-activated K conductance which participates in repolarization to a different degree in each cell type.

266 SOME CONNECTIONS OF THE OCTAVOLATERALIS AREA IN THE BOWFIN, AMIA CALVA. C. A. McCormick* (SPON: T. J. Neary). Div. Biol. Sci's., University of Michigan, Ann Arbor, Michigan 48109.

In adult specimens of the bowfin, Amia calva, either the anterior or posterior lateral line nerve was sectioned proximal to its ganglion. Following survival times of 8 to 17 days, the brains were processed by the Fink-Heimer method. After entering the brain, both nerves form ascending and descending bundles which terminate in the portion of the octavolateralis area designated by Pearson as nucleus medialis. Nucleus medialis consists of four cell types. Purkinje-like cells are situated immediately ventral to the cerebellar crest, and their dendrites have either a vertical or horizontal orientation. Interspersed among the Purkinje-like cells and ventral to them are small granule-like cells. Small fusiform cells and large polygonal cells are also found ventral to the Purkinje-like cells. Both lateralis nerves terminate ipsilaterally throughout nucleus medialis. Terminal degeneration is also found in the ipsilateral eminentia granularis of the cerebellum. No primary fibers ascend to the midbrain. Lesions of the glossopharyngeal nerve reveal no terminals in the octavolateralis area. Injections of HRP into the torus semicircularis indicate that the major projection from the octavolateralis area is from the Purkinje-like cells. Approximately 350 of these cells were labelled; 90% were located contralaterally. A few cells in nucleus medialis ventral to the Purkinje-like cells were also labelled bilaterally. This research was supported in part by NIH Grant 2 R01 NS 11006.

267 ORGANIZATION OF THE DORSAL COCHLEAR NUCLEUS IN MAN. J.K. Moore* (SPON: W. Wiederholt). Dept. Neurosci. and Neurobiol. Unit, Scripps Inst. Oceanog., UCSD, La Jolla, CA 92093.

The dorsal cochlear nucleus (DCN), generally described as vestigial in man, is in fact a well developed part of the human cochlear complex, being relatively larger in man than in the cat (Moore and Osen, in prep.). It differs significantly, however, in its internal cytological organization from that of the cat and other lower mammals. In the cat and prosimian primates, as in most mammalian species, the DCN exhibits a pattern of synaptic organization with features common to laminar cortical structures, a pattern oriented around a layer of radially oriented efferent neurons, the pyramidal or fusiform cells, which receive spatially segregated synaptic input from extrinsic fiber systems and intrinsic cortical neurons, including a prominent layer of granule cells. This laminar pattern of organization is completely lacking in the human DCN.

The alteration in cytoarchitecture in the human DCN is the outcome of a progressive loss of lamination observed in a phylogenetic series of primates. This loss is incipient in prosimians in which there is an apparently incomplete migration of the DCN granule cells, with retention into adult life of an extensive embryonic or external granular layer in addition to the normal internal granular layer. In both ceboid and cercopithecoid monkeys, granule cells are entirely subependymal: in cercopithecoids, this external granular layer is considerably reduced in volume. Associated with failure of migration and reduction of the granule cell population is a shift in the position of the pyramidal neurons to the central region of the nucleus. In the ape (gibbon) and in man, only vestiges of the molecular and granular layers remain. Thus in hominoids, the entire dorsal nucleus is equivalent to the central region of other mammals, with a large population of pyramidal cells oriented longitudinally, parallel to cochlear fibers, rather than radially across them. It remains a matter of speculation as to how changes in cytoarchitecture and loss of internal circuitry affect the function of the DCN in man.

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268 FACILITATION OF TONIC IMMOBILITY BY STIMULATION OF THE VAGINAL CERVIX IN THE RAT. A. N. Naggar* and B. R. Komisaruk (SPON: Benjamin Natelson). Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

Stimulation of the vaginal cervix with a plastic rod induced tonic immobility ("animal hypnosis") in response to inversion combined with brief manual restraint, in otherwise insusceptible female rats. Mean duration of the response was 14.8±1.9 (s.e.m.) seconds. In a second experiment, estradiol benzoate administration potentiated this effect somewhat. Short latencies to immobilization (≤5 seconds) were observed in at least two of the five trials in 6 of 6 EB treated animals, while only 2 of 6 oil treated controls met this criterion. Duration of tonic immobility was not significantly affected by EB treatment. This study supports the hypothesis that tonic immobility may utilize a neural system which is common to lordosis, the immobile mating posture of the female rat which is also facilitated by cervical stimulation and by estrogen.

269 STRUCTURE AND FUNCTIONAL RELATIONS: A MORPHOLOGICAL AND QUANTITATIVE ANALYSIS OF THE DEVELOPING MESENCEPHALIC NUCLEUS OF V IN BIRDS. C. H. Narayanan and Y. Narayanan*. Dept. of Anat., Sch. Med., New Orleans, LA 70119.

In order to understand structure-function relations during ontogeny between the neurons of the mesencephalic nucleus of V and jaw muscles, changes in cell morphology at the EM level and quantitative estimates based on cell number and cell size of the developing mesencephalic nucleus were investigated in white peking duck embryos. Frequency of oral activity (beak-clapping) from onset to hatching were recorded in embryos of the duck, chick and quail at one day intervals. Jaw movements showed a five-fold increase in the chick and quail, and a twelve-fold increase in duck embryos close to hatching. Estimates of cell number were determined from serial sections of brains stained with hematoxylin, eosin and orange-G. Cell loss amounting to more than 65% in normal development was observed between 6 and 16 days of incubation in the quail; 9 and 20 days in the chick, and 10 and 26 days in the duck.

Electron microscopic examination of the cells of the mesencephalic nucleus of the duck embryo which we have studied in detail, revealed significant developmental changes in their morphology and have been classified for the sake of convenience into three periods extending from the early appearance of the cells to hatching: An early phase, from 6 to 12 days, a mid-incubation period, 13 to 18 days; a last phase, from day 19 to hatching. The cells are recognizable as early as 6 days and are irregularly round or oval with very few cytoplasmic processes and an eccentrically placed nucleus which is more electron dense than in the adult. Growth of the cell continues through this phase and at the end of day 12 there is a reordering of cytoplasmic organelles with increasing basophilia of the peripheral cytoplasm. In the mid-incubation period, axons with neurofilaments and neurotubules are observed. Nissl granules appear in the periphery. The last phase is marked by an increase in the number of nerves associated with the cell soma. Coated vesicles appear to be budded off from the hypertrophic golgi. There is a greater accumulation of Nissl and a tremendous upsurge of neurofilaments and neurotubules, and myelination of the nerves. However, synapses associated with the neurons or its processes are absent at any stage until hatching, and suggested: a) the survival of neurons depends on the establishment of peripheral connections only; b) that mechanisms involved in the regulation of both quantitative and qualitative aspects of jaw movements are effective only after hatching. Supported by U.S.P.H.S. Grant DE04258-02.

270 EFFECT OF PREOPTIC LESIONS ON BEHAVIORAL THERMOREGULATION IN TWO TELEOSTS. D.O. Nelson* (SPON: J.E. Heath). Dept. Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

Fish can regulate their internal body temperature using physiological and behavioral responses. Behavioral thermoregulation consists of coordinated whole animal activity to create an environment of optimal temperature. Presence of central receptors involved in thermoregulation in fish has been demonstrated but not well localized previously.

Sunfish (Lepomis cyanellus) and goldfish (Carrassius auratus) were acclimated to 5, 15, or 25°C and then placed in a horizontal temperature gradient ranging from 2 to 30°C and position at various temperatures recorded over time. Fish avoided high and low extremes while spending large proportions of time in water near their acclimation temperature. Internal body temperature recorded with an implanted thermocouple remained relatively constant. After lesions were placed in medial and lateral preoptic regions in the forebrain, fish distributed themselves in the gradient spending nearly equal amounts of time at all temperatures available. Body temperature fluctuated significantly.

It is suggested that central temperature reception and integration is confined to discrete areas in the fish similar to that of higher vertebrates. Electrophysiological investigation of cells in this region is in progress.

271 HISTOFLUORESCENCE STUDY OF THE MONOAMINE-CONTAINING NEURONS IN THE BRAIN OF THE SUNFISH, Lepomis gibbosus. A. Parent, L. Dubé*, M.R. Braford Jr. and R.G. Northcutt. Lab. Neurobiol., Fac. Med., Laval Univ., Quebec, Canada and Div. Biol. Sci's, Univ. of Michigan, Ann Arbor, MI 48109.

A study of the morphological organization of the monoamine (MA)-containing neurons in the brain of the sunfish was undertaken by means of the Falck-Hillarp paraformaldehyde histofluorescence method (Falck et al., '62). Numerous MA perikarya are present in this teleostean brain. By far the largest number of MA cell bodies occur in the hypothalamus especially along and within the ependymal wall of the lateral and posterior recesses of the third ventricle. They consist of small bipolar cells displaying a strong yellow-green fluorescence. One of their processes protrudes into the lumen of the ventricle whereas the other contributes numerous closely packed MA terminals that form "islands" of highly fluorescent material distributed in various areas of the medial hypothalamus. In the brain stem a small group of large sized catecholamine (CA) neurons occurs in the dorsal portion of the isthmal tegmentum, partly within the periventricular gray. These closely packed neurons often emit one short and thick process that bifurcates close to the cell body. More caudally a few medium sized and elongated CA type neurons are scattered within the central portion of the lower medulla oblongata. These neurons are embedded in a mesh of fine varicose CA processes. In addition serotonin (5-HT) type cell bodies are found in the raphe region from caudal midbrain to upper medullar levels. Numerous MA axon terminals are also concentrated in various areas of the sunfish brain such as: the dorsolateral quadrant of the lower medulla, the lateral edge of the isthmal tegmentum, the valvula cerebelli, the optic tectum and the torus semicircularis. At diencephalic levels CA terminals are present in nucleus glomerulosus and more abundantly in the whole preoptico-hypothalamic complex, especially the inferior lobe. The entire telencephalon also receives a strikingly massive and complex MA innervation. MA fibers (mainly CA) ascend within the basal forebrain bundles and ramify more abundantly in the dorsomedial and the ventral part of the dorsolateral telencephalic territories. The profuse telencephalic CA innervation in the sunfish could arise from the CA neurons of the isthmus which appear strikingly similar to the locus coeruleus of reptiles and mammals.

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272 THE DISTRIBUTION OF GANGLION CELLS IN THE RETINA OF THE CALIFORNIA HORNED SHARK(Heterodontus francisci). Ellengene H. Peterson and Michael H. Rowe. Dept. Anat., Univ. Chicago and Dept. Anat., Univ. Ill. Med. Center, Chicago, Ill. 60680.

The distribution of retinal ganglion cells was examined in cresyl violet stained whole mounted preparations of the retina of the California horned shark. A consistent observation was the presence of two distinct ganglion cell layers. In addition to the conventional ganglion cell layer located just external to the fiber layer, there was a second ganglion cell layer located between the conventional ganglion cell layer and the inner nuclear layer. The ganglion cells closest to the fiber layer have been called the inner ganglion cell layer (IGCL), and those farther from the fiber layer the external ganglion cell layer (EGCL). The degree of separation between the two layers was not constant, being greater in regions of high ganglion cell density. Ganglion cell density in the IGCL ranges from approximately 0-700 cells/mm^2, and in the EGCL from about 0-160 cells/mm^2. Isodensity maps of the IGCL show an elongated region of high ganglion cell density (visual streak) extending from nasal retina to temporal retina, and oriented so that its long axis follows approximately the horizontal axis of the eye. The ganglion cell distribution in the EGCL is qualitatively similar to that in the IGCL, but the overall densities are lower, and the density gradients are much weaker. Ganglion cells in the visual streak of the IGCL range in soma diameter from 9-28 µm with a mean of 14.4 µm. Ganglion cells in corresponding regions of the EGCL range from 10-27 µm with a mean of 17.4 µm. Both distributions are unimodal. In non-streak regions of the retina the size differences between cells of the two layers seem less pronounced. In addition to the ganglion cells in the IGCL and the EGCL, many 'displaced' ganglion cells were observed with somas located in the inner nuclear layer. These cells ranged in size from 10-35 µm and appeared to have a relatively uniform retinal distribution.

273 NEURAL CONTROL OF THE PROBING PHASE OF BURROWING IN BIVALVE MOLLUSCS. David J. Prior, Sch. Biol. Sci., Univ. of Kentucky, Lexington, Kentucky 40506.

The behavioral responsiveness of an animal to a particular stimulus often varies drastically. As a portion of a study of variation in responsiveness, the control of the probing phase of burrowing behavior in bivalve molluscs has been examined. The mechanics of burrowing in several bivalves have been previously described (Trueman, 1967). Cyclical burrowing in bivalves includes probing the substrate with the tip of the foot, expansion of some portion of the foot to establish an anchorage and finally pedal retraction, which due to the anchorage, draws the animal down into the substrate. The probing phase of this sequence consists of alternating extensions and retractions of the distal portion of the foot. The frequency of probing varies considerably between species and between individuals within a species but usually is in the range of 1 probe/1-5 sec. Simultaneous recordings of foot movement and pedal nerve activity were made from preparations consisting of the foot, pedal ganglion and cerebral ganglia. Stimulation of the cerebral-pedal connectives resulted in prolonged series of cyclical probing movements and corresponding cyclical bursts of efferent activity in the pedal nerves. The mean probing frequency recorded from this sort of preparation is 1 probe/1.8 sec. which is well within the range observed in intact animals. The major bursts of pedal nerve activity corresponds with the retraction phase of probing. During probing neither tactile stimuli applied to the surface of the foot nore electrical stimulation of the pedal nerves will disrupt the cyclical efferent output. The probing bursts can be ellicited from isolated pedal ganglia and do not differ in frequency from those observed in the narrowly intact preparations. Hence, the motor program for probing is entirely within the pedal ganglion. Intracellular recordings have been made from both retraction motor cells (RMC) and extension motor cells (EMC) in the pedal ganglion. During foot probing the RMCs generate bursts of spikes as a result of cyclical excitatory synaptic input. The antiphasic bursts generated by the EMCs occur due to rebound activation following cyclical inhibitory synaptic input. The EMCs show a greater tendency for rebound activation than do the RMCs. Supported by NSF Grant BMS74-15217-A01.

274 VISUAL DISCRIMINATIVE PERFORMANCE IN THE TURTLE FOLLOWING LESIONS OF FOREBRAIN VISUAL STRUCTURES. Tony Reiner* (Spon. H.Karten). Dept. Anat., S.U.N.Y. at Stony Brook, Stony Brook, N.Y. 11794.

An ascending tectofugal visual pathway has been described in mammals, birds and reptiles. In mammals and birds, this pathway has been shown to play a major role in visual functions (Snyder and Diamond, BBE, 1968 and Hodos and Karten, Exp. Br. Res., 1966 and JCN, 1970). In reptiles, however, information about the functional role of the tectofugal pathway is meager. The present work was carried out with turtles (Chrysemys picta picta). In turtles the tectofugal visual pathway consists of the following serially connected structures: the tectum, nucleus rotundus thalami, and the dorsal ventricular ridge of the telencephalon (DVR) (Hall and Ebner, BBE, 1970 and JCN, 1970). The role of the tectofugal pathway was examined in turtles by studying the effects of lesions placed in nucleus rotundus or the DVR upon visual discriminative performance.

One group of turtles was preoperatively trained to criterion on a simultaneous intensity discrimination (0.4 log unit difference), while a second group was trained to criterion on a simultaneous pattern discrimination (horizontal vs. vertical stripes). Half the animals in each group received bilateral lesions of nucleus rotundus, while the other half received lesions of the DVR. Turtles were retrained to criterion on the same discriminative problem that they had been trained on preoperatively. Once a turtle had either reattained criterion, or demonstrated an inability to do so, training was terminated. Turtles were then anesthetized, perfused and the extent and locus of the lesions reconstructed from cresyl violet stained frozen sections.

The results were: 1) Turtles with extensive destruction of nucleus rotundus were unable to relearn their discriminative task, whether intensity or pattern. 2) Turtles with extensive DVR damage were also impaired in their postoperative discriminative performance. However, these turtles did relearn. 3) Turtles with only slight damage to either the DVR or nucleus rotundus showed good retention of their discriminative tasks.

The results indicate that the forebrain components of the tectofugal visual pathway play a major role in visual functions in the turtle. However, further research is necessary in order to determine the basis for the greater deficit following nucleus rotundus lesions than DVR lesions.

275 THE AVIAN TECTOFUGAL VISUAL PATHWAY: PROJECTIONS OF ITS TELENCEPHALIC TARGET, THE ECTOSTRIATAL COMPLEX. Teresa C. Ritchie and David H. Cohen. Dept. Physiol., Sch. Med., Univ. of Virginia, Charlottesville, VA 22901.

The avian visual system includes two major ascending pathways, a thalamofugal pathway homologous to the mammalian geniculo-striate system and a tectofugal pathway corresponding to the tecto-thalamo-extrastriate system. The tectofugal pathway involves a topographic retinal projection upon the optic tectum with efferents from various levels of stratum griseum centrale projecting upon subdivisions of the thalamic nucleus rotundus. Rotundal subdivisions project to subdivisions of ectostriatum, a prominent telencephalic nucleus, which then sends fibers to nearby neurons forming a peri-ectostriatal belt (E_p). This report describes further projections of this system in the pigeon.

First, a series of electrolytic lesions were made in the ectostriatal complex including E_p, and resulting degeneration was studied with selective silver methods. The complex projected upon a longitudinally oriented cell column in lateral neostriatum intermedium (NIL), degenerating axons streaming dorsolaterally and caudally from the lesion to terminate throughout this column. The terminal field extended rostrocaudally from caudal ectostriatal to caudal archistriatal levels. Degenerating fibers were also followed from the lesion, through the lamina medullaris dorsalis, to a cell group in archistriatum intermedium dorsalis (AID).

Second, to identify the cells of origin of these two projections, horseradish peroxidase was injected into the terminal fields and the material processed after 1-3 days by conventional procedures. Injection of the NIL field labelled cells in the dorsal E_p, extending as dorsally as lamina hyperstriatica. Labelled cells following injection of the AID field were located ventrocaudally to ectostriatum, a region where E_p forms a caudal cap around ectostriatum.

Third, electrophysiological experiments were conducted on urethane-anesthetized birds with stimulating electrodes on the optic papilla. Field potentials evoked by papillary stimulation were recorded with 4M NaCl micropipettes and computer averaged. Localized negative fields with peak latencies of 18 and 13 msec were found in regions corresponding to the NIL and AID terminal fields respectively.

These findings confirm that ectostriatum projects upon E_p. E_p has at least dorsal, lateral and ventrocaudal subdivisions, the dorsal division projecting upon a region of NIL and the ventrocaudal division projecting upon a subnucleus of AID. Electrophysiological results confirm that both these terminal fields receive visual input.

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276 THE SYNAPTIC ORGANIZATION OF OPTIC AFFERENTS TO THE NORMAL GOLDFISH TECTUM. John T. Schmidt. Develop. Biol. Div. Nat'l Inst. for Med. Res., Mill Hill, London,NW7 1AA England

Potentials in the tectum of large (12-20cm) goldfish, evoked by stimulation of the optic nerve, were recorded extracellularly with double barrelled electrodes (DC,saline,3MOhms; AC, Woods Metal-Pt., 0.1MOhm). Four fiber groups (m_1,m_2,m_3,m_4) were recorded at latencies of approximately 3,4,6 and 8 msec after stimulation (Conduction velocities of approximately 5,4, 2.5 and 2 m/sec). The same four groups were recorded from the optic nerve when the tectum was stimulated. The fastest of these (m_1) was not followed by a postsynaptic wave. Fiber groups m_2,m_3 and m_4 gave rise to postsynaptic potentials, which following computation of their second spatial derivatives with depth, were found to have current sinks at depths of approximately 90-110 µm, 110-200 µm and 200-300 um respectively. Thus the fastest conducting optic fibers make their synapses most superficially, the opposite of the arrangement in the frog tectum(Chung,Keating and Bliss,Proc.Roy.Soc.B187(1974)421). These postsynaptic waves fatigued at repetitive stimulus rates of 20-40 per second, and in twin pulses at interstimulus intervals of 10-15 msec; and were reversibly blocked by topical application of Nembutal. The fiber potentials, however, were virtually undecremented under these conditions.

To compare these electrophysiological findings with the actual anatomy, the cobalt procedure (Tyrer and Bell,Brain Res. 73(1974)151) was used to visualize the profiles of the optic fibers in the various tectal laminae. In contrast to previous reports using radioautography, four discrete layers were found. The thick, dense superficial band and its thin superficial satellite band (90-200 µm) were noted as in radioautographs. In addition, there were two deeper bands of sparse optic fiber innervation at approximately 240 µm (Central Grey) and approximately 350 µm (Deep White). These two deep bands appear to correspond to the m_4 fiber group. The dense superficial band apparently contains both the m_2 and m_3 fiber groups, the m_2 just superficial to the m_3. The fastest fiber group (m_1), which had no presynaptic wave associated with it, was of maximum amplitude in the superficial 100 µm and probably represents efferent cell bodies with fibers projecting back through the optic nerve to the retina. Filled cell profiles could often be seen in the superficial laminae with the cobalt technique.

277 THE HISTOGENESIS OF THE GOLDFISH RETINA. S. C. Sharma and F. Ungar*. Dept. Ophthal., N.Y. Med. College, New York,N.Y.10029

The order of production of retinal cells was studied in the embryos of the teleost, Carassius auratus, using ^{3}H-thymidine autoradiography. Cells become post-mitotic first in the fundus of the neuroepithelium and continue longest at the margin. In the fundus the cells whose nuclei will reside in the inner region of the retina, near the vitreous, stop dividing before the cells whose nuclei will reside near the outer region of the retina. The sequence of cells becoming post-mitotic in the fundus zone is such that cells destined to become ganglion cells are produced first, followed by amacrine cells, bipolars and Muller's cells. The last cells to stop dividing in the fundus differentiate as horizontal cells followed by receptors.

In the later stages of development, no labelled nuclei were found in the fundus zone; however, cells near the margin of the retina were labelled. The label incorporation in the marginal areas suggests that the retina increases in area by addition of new cells at the retinal margin.

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278 GROUP SIZE DEPENDENT DISPLAY OF SEXUAL BEHAVIOR IN THE SQUIRREL MONKEY. C. J. Sherry+ and F. A. Rowe. (Illinois Institute of Technology, Chicago, Ill. (+Current address Texas A & M Univ. College Station, Tx. 77843)

Observations of social behavior of infrahuman animals is usually obtained from paired encounters of animals or in larger social groupings. Primate social behavior in general and sexual behavior in particular are usually obtained under one of three conditions: 1) natural habitats with large naturally occurring social groupings (Thorington, R.W. in Rosenblum, L.A., The Squirrel Monkey, Academic, 1968); 2) seminatural habitats in a laboratory (DuMond, F.V. ibid); or 3) paired encounters in a laboratory (Mason, W. A. Primate Behav. 1971, 2, 107). Little data has been obtained about the effect of group size, when the group is smaller than the naturally occurring social group on the display of social and/or sexual behavior. The squirrel monkeys used in this experiment were housed individually in a room with temperature and humidity controlled and food and water available ad librium. The light dark cycle was adjusted to 12 hours on, 12 off. The monkeys were observed for 15 minutes a day on four consecutive days by two observers in each social condition. They were scored for the display and duration of various social behaviors. When observing the male squirrel monkeys individually (A), in pairs (P), groups of three (T), or four (Q), we found that sexual behaviors such as mounting occurred more often in larger groups of animals (P X=0), (T X=0.98, S.D.=2.18) (Q X=0.88, S.D.=1.71). Other sexually related social behavior also tended to occur more often in larger groups. For example, penis display, a socially aggressive signal, tended to occur more often in groups of three or four than in pairs (P X=1.69 S.D.=4.03), (T X=3.79 S.D.=4.32), (Q X=3.13 S.D.=4.32). Masturbation, an individually oriented sexual behavior, also tended to occur more often in larger groupings (A X=0), (P X=0.57 S.D.=1.28), (T X=0.83 S.D.=1.26), (Q X=1.38 S.D.=3.10). Autofellatio, another individual sexual behavior also occurs more often in larger groups (A, P, T X=0), (Q X=0.58 S.D.=1.08). Grooming did not occur in groups of three or four, but it did occur in pairs (X=0.57 S.D.=1.36). Marking (i.e. rubbing urine on the hand or foot and then rubbing it on the perch) did not occur in animals observed alone, in pairs, or groups of four, but it did occur in groups of three (X=0.58 S.D.=0.82).

It will be important to determine why group size effects sexual behavior, in general, and individually directed sexual behaviors, in particular.

279 HYPERPOLARIZING POTENTIALS INDUCED BY Ca-MEDIATED K-CONDUCTANCE INCREASE IN HAMSTER PARASYMPATHETIC NEURONS. T. Suzuki* and K. Kusano. Dept. Biol., IIT, Chicago, IL. 60616.

Three types of hyperpolarizing potentials in the hamster submandibular ganglion cells were analysed by modifying ionic constituents of the perfusion saline or by applying several drugs: (1) the spike induced hyperpolarizing after-potential (S-HAP), (2) the hyperpolarizing (H-) phase of postsynaptic potential and (3) the spontaneous transient hyperpolarizing potential (HP). Experiments were carried out in vitro. All these potentials reversed polarity at membrane potential (E_m) between -75 and -85 mV, which was close to the K-equilibrium potential. The average resting potential of the ganglion cells was -53 mV (range: -40 - -75 mV). In most neurons action potentials were suppressed by 10^{-7}M tetrodotoxin (TTX) but were resistant in others even to 10^{-6}M TTX. Action potential overshoot increased slightly in raised $[Ca^{2+}]_o$ and decreased in low $[Ca^{2+}]_o$. The S-HAP was due to the K-activation but it could be separated into two components: an initial component due to delayed rectification and a late component. The late component was Ca^{2+}-sensitive and it was easily suppressed by lowering $[Ca^{2+}]_o$, or by Mn^{2+}. This component was enhanced by increasing $[Ca^{2+}]_o$, TEA, caffeine and dibutyryl cyclic AMP. Perfusion of Cl^--free saline, substituted with acetate, reduced membrane potential slightly but it did not modify the S-HAP. Application of depolarizing pulses to TTX-sensitive neurons also induced hyperpolarizing after-potentials, similar to the S-HAP. The postsynaptic potential (PSP) was a monophasic depolarizing (D-) potential in some neurons, but in others the D-phase was followed by a small H-phase in control saline. The reversal potential of the D-phase was +1.4 ± 4.9 mV SD (n=5). Perfusion with: K^+-free, 2 mM caffeine- or 2 mM dibutyryl cyclic AMP-containing salines increased the H-phase of the PSP. An increase of membrane conductance was sometimes observed during the H-phase. Spontaneous transient HP occurred in some neurons at irregular intervals (e.g., 16 mV in amplitude, 3 sec in duration, ca. 0.3 Hz at -52 mV E_m). HP was insensitive to TTX but was suppressed by Mn^{2+}. Caffeine induced rhythmic HP's in many neurons; often followed by repetitive spiking. Membrane resistance decreased during HP, then increased slightly before returning to the basal level. In a few neurons which had a low E_m, caffeine induced a long-lasting hyperpolarization. These membrane potential-dependent phenomena appeared to be induced by the Ca-activated K-conductance increase. (Supported by the US PHS Grant NS-12275).

280 EFFECTS OF SEPTAL LESIONS ON THE AGGRESSIVE BEHAVIOR OF THE WESTERN FENCE LIZARD, SCELOPORUS OCCIDENTALIS. Robert S. Tarr*, (SPON: Michael Patterson). Dept. of Physiol., Chicago Coll. of Osteopathic Med., Chicago, Ill. 60615.

The effects of septal lesions on interspecies and intraspecies aggressive behavior was examined in a two part study. Part I was the effects of septal lesions on reactivity and "emotionality". 16 animals were lesioned:8 septal, 3 outside septum and 5 sham. Each animal was then individually rated (blind) by two observers on a rating scale for light touch, handling and mildly noxious stimulation (pinch). Animals were classified by total average score into 3 classes (low, middle, high) of reactivity and emotionality. Comparison of lesion site with behavioral rank was made. Septal lesioned animals were widely distributed in all 3 classes of reactivity and emotionality, did not average higher scores than the non-septal and sham lesioned animals and showed no signs of a "septal syndrome".

Part II was a study of intraspecies aggressive behavior. Groups of animals were observed in a large (100 sq. ft) seminatural environment. Aggressive behaviors (dominance, subordinance, challenge and assertion display, submissive posture, location preference in the territory and response to display) were recorded for each animal. Animals were removed, lesioned and returned for comparison with prelesion behavior. 12 animals received septal lesions, 5 were given non-septal lesions and 9 were sham lesioned. 20 control animals, (reported earlier Physiology and Behavior, Vol.18(6),1977) were also removed and only anesthetized then returned. The 20 control animals, the 9 sham lesioned animals and 4 of the non-septal animals showed no change in aggressive behavior upon reintroduction. 9 septal animals showed no change. 3 of the septal lesioned animals showed a partial loss of display, flight and submissive behavior. This is in contrast to amygdaloid lesions in these animals which consistently produce a near total loss of intraspecific aggressive behavior (referenced above).

281 EFFERENT PROJECTIONS OF THE SEPTAL AREA IN THE TEGU LIZARD. T. J. Voneida and C. M. Sligar*. Dept. Neurobiol., Northeast. Oh. Univ. Coll. Med., Kent, OH 44240 and Dept. Anat., Sch. Med., CWRU, Clev., OH 44106.

A mixture of {^{3}H} proline-leucine concentrated to 25 µCi/µl was pressure injected into the septal area of 10 adult lizards. Injection rates varied from 0.055-0.35 µl/hour. Following survival times ranging from 3 days to 2 weeks, labeled pathways were traced dorsally into the ipsilateral medial cortex and caudally via ipsilateral stria medullaris thalami and medial forebrain bundle. Labeled fibers in stria medullaris enter the medial habenular and dorsomedial thalamic nuclei; those in the medial forebrain bundle appear to terminate within the hypothalamus as follows: medial preoptic, dorsal and lateral hypothalamic areas; anterior, posterior and paraventricular nuclei; medial mammillary nuclei. Mesencephalic projections (pre-rubral field, central grey and substantia nigra) appear not to result from septal injections, but were consistently noted when injection sites included nucleus accumbens. (Supported by Grants MH-07051 and EY-04090 from the National Institutes of Health).

282 SOMATOSENSORY PROJECTIONS TO THE THALAMUS IN RANID FROGS. Walter Wilczynski, Timothy J. Neary, and Michael L. Andry*. Neurosci. Prog. and Div. Biol. Sci., Univ. of Mich., Ann Arbor, MI, 48109.

Electrophysiological studies (Vesselkin, et al., BB&E 4:295, 1971) indicate that there is an extensive somatosensory representation in the thalamus of anurans. In order to determine more precisely the pattern of termination of ascending somatosensory input, we have engaged in a series of both anterograde autoradiographic and retrograde HRP experiments in ranid frogs. No evidence for a direct spinothalamic projection could be seen following injections of up to 4 uCi of a [^{3}H]-proline-leucine mixture into the spinal cord proper. When injections were placed near the obex region, however, an extensive thalamic projection was observed, primarily contralateral to the injection. Label was present in an intermediate portion of the anterior nucleus and in the medial half of the posterior nucleus. In addition, label was present over the entire rostrocaudal extent of the ventral thalamus, excepting a dorsolateral area rostrally. After large HRP injections into the rostral thalamus, HRP-positive cells were found in the caudal medulla. These labelled cells were present in the reticular formation ventrolateral to the hypoglossal nucleus, and in a perisolitary band of cells lying dorsal and lateral to the solitary tract. Of 112 cells counted in two cases, 26 were found in the reticular area, with 15 of these ipsilateral to the injection sites. Eighty-six cells were found in the perisolitary band, with 73 of these contralateral to the injection sites. No labelled cells were seen in the spinal cord proper.

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DEVELOPMENT AND AGING

283 INTERRELATIONS OF SENSORIMOTOR CORTICAL AND SUBCORTICAL CIRCUITS SUBSERVING CONTACT PLACING IN THE KITTEN. Vahe E. Amassian, Richard Ross and Birgit Zipser. Dept. of Physiology, SUNY, Downstate Medical Center, Brooklyn, N.Y. 11203.

Contact placing (CP) of the forepaw recovers after either extirpation of contralateral sensorimotor cortex (J.Physiol. (1972) 230: 55-56P) or after a midbrain decerebration prior to the fourth postnatal week. However, forepaw CP is abolished by transecting the spinal cord at C1, the kitten being maintained on artificial respiration. In decerebrate kittens, a radiofrequency lesion of the Vestibular N. ipsilaterally abolishes forepaw CP (J.Physiol. (1977) 266: 97-98P). Participation of the Vestibular complex in CP could account for its facilitation (eg, an increased probability and reduced latency of CP) when the kitten is moved to the placing apparatus instead of P→K. After recovery from a SM cortical lesion, the 'stand alone' capacity of the subcortical circuitry is secondarily lost when the kitten ages, CP probability gradually diminishing at 1-2 months until it resembles the effect of an initial cortical removal at 5 weeks.

Starting with the second week, if corticofugal output is acutely removed by cooling SM cortex, forepaw CP is temporarily lost to lateral but not to dorsal stimulation; cooling during the first week is usually ineffective suggesting that SM cortex does not acquire an important role in a behavior-lateral CP until the second week (J.Physiol. (1976) 263: 144-145P). This influence is probably mediated by PT neurons; thus, cooling the rostral bulbar pyramid of 2-3 week old kittens through a probe implanted via the cerebellum abolished lateral CP first contralaterally, the forelimb adopting an extended posture. When placing failed, the early SI cortical response to contralateral forelimb stimulation was unaltered in amplitude or was reduced suggesting that cooling had spread to the medial lemniscus. Cooling with the probe slightly withdrawn spared lateral placing, but reduced the SI response. The possibility that, early in development, PT neurons might mediate lateral CP tonically rather than by dynamic responses to contact was supported by the finding, at 3-4 weeks, that thalamic cooling adequate to block the early SMI responses to stimulation contralaterally of the forelimb and cerebellum, respectively, did not abolish lateral CP.

Electrophysiological indices of maturation were sought that might relate to the above findings. In a sample of 100 individual, antidromically labelled PT neurons, the shortest conduction time from bulbar pyramid to motor cortex fell from 32 to 10 to 5 msec in the first, second and third weeks respectively. During the second week, the fastest PT neurons conduct at 3 m/sec, ie, they are unmyelinated when they first function in CP. Significantly, resting discharge first becomes prominent in the second week, providing a basis for the tonic PT influence on CP. Aided by NS11219.

284 RELATIONSHIP OF SIZE AND NEURONAL DENSITY OF CAUDATE NUCLEUS TO THE NUMBER OF DOPAMINERGIC NEURONS IN DIFFERENT MOUSE STRAINS. A CENTRAL MODEL OF NEURON-TARGET ORGAN INTERACTION. H.A. Baker*, T.H. Joh, and D.J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021.

Differences in the activity of brain tyrosine hydroxylase (TH) between 2 inbred mouse strains, Balb/cJ and CBA/J are due to differences in the number of immunocytochemically demonstrable TH-containing dopaminergic (DA) neurons in the midbrain (Ross et al Nature, 264.654, 1976). We sought to determine if the strain-dependent differences in DA cell numbers were associated with any morphological or biochemical variations in a target of DA neurons, the caudate nucleus (CN). As previously found, TH activity in the substantia nigra (SN) and CN of Balb was greater than in CBA mice. TH activity in the SN of Balb (2.13±.08 nmol/Dopa/hr/SN) was 23% higher than in CBA mice (1.66±.04) and 19% greater in the CN of the Balb (734.4±25.11 nmol Dopa/gm CN/hr) than in CBA mice (601.9±18.3) ($P<.01$). The total number of midbrain neurons immunocytochemically stained for TH in Balb was 16% greater than in CBA mice (7,404 vs. 6,250 cells). No strain difference in TH activity or cell number was observed in the locus coeruleus. The CN volume in Balb was 16% larger than in the CBA mice (8.19±0.28 vs. 6.92±.23 mm^3 $P<.05$). Since the neuronal density (87,314± 6,335 in Balb vs. 89,236±6,637 cells/mm^3 in CBA) did not differ, the total number of CN neurons was greater in Balb than in CBA mice. In CN, choline acetyltransferase (CAT) activity was identical for both strains. However, glutamic acid decarboxylase (GAD) activity, was 19% lower ($P<.01$) in the CN of Balb (12.22±.75 nmol CO_2/gm CN/hr) than in CBA mice (15.05±.74). In contrast, in SN, GAD activity was the same in the two strains. CAT activity in Balb was 27% higher than in the CBA mice (71.04±6.0 nmol ACh/SN/hr and 52±4.4 respectively $P<.05$). We conclude that strain-dependent differences in numbers of DA neurons directly correlate with the size and number of neurons in the terminal field. The finding suggests an interaction between cell number and target organ size in the CNS, as in the periphery. Biochemical mechanisms involving cholinergic and gabergic systems important in the interaction between the SN and CN may compensate to provide for neurological normalcy in these strains.
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285 SYNAPTIC MODIFIABILITY IN MIDDLE-AGED AND SENESCENT RATS. C. A. Barnes. Dept. of Psych., Dalhousie Univ., Halifax, N. S., Canada, B3H 4J1

The perforant path - granule cell synapse in the hippocampus shows a long-lasting type of increased effectiveness after brief episodes of high-frequency stimulation. Since the synapse is a favored candidate for the site of information storage in the nervous system, it was of interest to determine whether there were any differences in the synaptic physiology of mature and very old organisms. Thirty-two middle-aged (16 months) and 32 old (34 months) hooded rats were tested on a complex spatial discrimination task on which the old rats showed retention deficits. These same rats were then prepared for chronic stimulation and recording of perforant path-granule cell responses. All electrophysiological testing was performed with the animals awake and unrestrained. With the stimulation parameters used in this study, electrical afterdischarge did not occur. After one burst of high-frequency stimulation, the fractional increase in the synaptic response, as well as the time course of decay of this increase over a period of one week, was virtually identical between age groups. When high frequency bursts were delivered at 24-hour intervals, however, a large difference in the retention of the elevated evoked response became apparent between age groups. Fourteen days after the final high frequency stimulation, the young rats continued to show the large increase in synaptic response, whereas the response in the old animals had decayed back to the initial baseline. If this physiological model bears any relation to learning in the natural situation, it predicts that the deficit which comes with age is in retention and not in initial learning.

The fractional increase of synaptic response was affected by the time of day in the young but not in the old rats, suggesting alterations of circadian rhythmicity in CNS functioning of the old rats. At the end of the study the effects of pentobarbital anesthesia on the synaptic response was measured. The size of the extracellularly recorded EPSP in both groups was reduced, however the reduction was significantly greater in the old animals. This suggests that extrapolations of electrophysiological data obtained from preparations using this anesthetic to awake animals is extremely tenuous, particularly when age comparisons are being made.

286 OBSERVATIONS ON THE DEVELOPMENT OF BRAINSTEM-SPINAL SYSTEMS IN THE NORTH AMERICAN OPOSSUM. J. Beals[1], J. Culberson[2], R. Dom[3] and G. Martin[4]. Dept. of Life Sciences, Otterbein College, Westerville, Ohio[1], Depts. of Anatomy, West Virginia Univ., Morgantown, W. Va.[2], Univ. of S. Carolina, Charleston, S.C.[3], and The Ohio State Univ., Columbus, Ohio[4].

The opossum's embryology makes it a potentially good model for studies of developing motor systems. The young are born 12 + days after conception and are carried in the mother's pouch for 90 days or more where they are accessible for experimental manipulation. Of particular interest is the fact that their hindlimbs remain immobile for a week or more after entrance into the pouch. We have begun studies with the opossum which were designed to gain insight into the development of brainstem-spinal connections and their possible correlation with the ontogeny of hindlimb activity.

By using the Fink-Schneider method on pouch young opossums with appropriate lesions (see C.M. Leonard, Brain Res., 53: 412-416) we have been able to demonstrate "immature" suprasegmental fibers in the marginal layer of the lumbar cord prior to the development of hindlimb motility. The electron microscope reveals that marginal axons are small and unmyelinated at such stage. By the time random hindlimb movements begin, "immature" brainstem fibers can be found in the expanding neuropil of the mantle zone. At that stage they can be demonstrated in most of the areas which contain such fibers in the adult and HRP studies show that they take origin from most of the cell groups giving rise to comparable fibers in the mature animal. Once the young are weaned and able to ambulate in an adult fashion, brainstem-spinal axons are mature in appearance (all techniques) and can be demonstrated by the Fink-Heimer method. Correlative electron microscopic studies are underway which are designed to address questions of developing synaptology in these systems.
(Supported by U.S.P.H.S. Grant NS-07410.)

287 NOCICEPTION: POSTNATAL DEVELOPMENT OF RESPONSIVENESS TO ANTICHOLINERGICS. C. T. Bennett and J. M. King. Neuropsychology Br., Experimental Medicine Div., Biomedical Laboratory, Chemical Systems Laboratory, APG, MD 21010.

Responses of rats (5, 10, 15, 20 and 25 days of age) to nociceptive stimuli were measured using the tail flick and hot plate tests. Following baseline measurements, the neonates received either saline (0.9%) or benactyzine (5.0 or 10 mg/kg) and tested 15 minutes later. In the hot plate test, 5 mg/kg benactyzine had no behavioral effect at any day tested. By 20 days of age, however, 10 mg/kg of benactyzine significantly reduced response latency in this procedure. In contrast to this is the effect of benactyzine on the tail flick behavior of the neonates. Beginning at 5 days of age, the low dose of benactyzine (5 mg/kg) significantly reduced tail flick latency. This effect was antagonized by physostigmine (0.3 mg/kg). The data clearly indicate a differential effect by dose, and by age, on the two tasks differing in motor complexity. These studies suggest that the functional development of the cholinergic system mediating nociception does not develop homogenously.

288 INTRACELLULAR LOCALIZATION OF ALPHA-FETOPROTEIN (αFP) IN THE DEVELOPING RAT BRAIN - AN IMMUNOCYTOCHEMICAL STUDY. Robert H. Benno* and Terence H. Williams. Dept. of Anatomy, University of Iowa, Iowa City, Iowa.

Alpha-fetoprotein (αFP) is a serum protein present temporarily in high concentrations in the serum of developing vertebrates, but absent in the adult. Molecules of αFP with specific estrogen binding properties exist in the developing rat brain and, on the basis of biochemical evidence, it has been hypothesized previously that αFP is present extracellularly in the developing brain. Whereas an appropriately timed action by estrogen on the developing rat brain appears to be both beneficial and necessary, it has been claimed that premature estrogenization may be deleterious, and that αFP serves to block this effect. The objective of this study is to localize αFP in developing brain to gain clues concerning its true role.

Thirteen, 15, 18 day fetal, 2 and 5 day postnatal and adult male and female Sprague-Dawley rats were perfused with Bouin's fixative and embedded in paraffin. Five micron sections were cut and processed for immunocytochemistry by the unlabeled antibody peroxidase-antiperoxidase technique.

Intracellular localization of αFP in the rat brain was noted in all animals aged from 15 days fetal to 5 days postnatal. Many recognizable neuronal cell groups contain αFP. These include some that have been shown to possess high affinity estrogen receptors. An interesting finding was discrete high intensity localization in some, but not all, of the cells lining the ventricles. Also, αFP was associated with meninges, choroid plexus, circumventricular organs and blood vessels. The localization of αFP in cells surrounding blood vessels in the brains of the 18 day fetal animals and the absence of similar staining in the 2 and 5 day postnatal animals suggested to us that maturation of the blood-brain barrier (BBB) prevents access of blood borne αFP into most brain areas. Persistent αFP localization observed in the circumventricular organs and choroid plexus up to postnatal day 5 can be explained by absence of the BBB in these areas. αFP was absent in the adult brain.

Two alternative hypotheses can be constructed to explain the observed immunocytochemical localization of αFP in developing rat brain:

(1) All neural cells may require αFP for some process associated with differentiation and/or migration.

(2) Only a particular population of cells, possibly estrogen sensitive cells, may require αFP.

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289 SOURCE OF NORADRENERGIC SPROUTING IN THE CEREBELLUM OF RATS TREATED NEONATALLY WITH 6-HYDROXYDOPAMINE. Ranbir K. Bhatnagar and Richard H. Schmidt*. Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Treatment of neonatal rats with subcutaneous 6-hydroxydopamine (6-OHDA) is known to produce an extensive, permanent degeneration of noradrenergic (NE) fibers in the telencephalon, but results in a marked regenerative sprouting of NE terminals in the cerebellum. It is not known if the locus coeruleus is the source of all or any of these sprouted fibers, as other NE cell groups also appear to contribute to the cerebellar innervation. This study was conducted to determine the regional distribution of locus coeruleus innervation to the cerebellum in normal and 6-OHDA treated rats.

Female rat pups were injected subcutaneously with 100 mg/kg 6-OHDA or vehicle on postnatal days 1 and 2. When 100 days of age unilateral electrolytic lesions (2mA for 10 seconds) or sham implacements were made in the locus coeruleus. Two and three weeks later the cerebellum was removed and dissected into anterior, middle and posterior regions as described in the abstract by Schmidt and Bhatnagar. Each region was assayed for synaptosomal NE uptake, dopamine β-hydroxylase (DβH) and endogeneous NE.

Ipsilateral to the lesion in vehicle control rats NE levels in the parietal cortex were reduced to 15% of sham control, while on the contralateral side NE was reduced by less than 25%. This, as well as histology, indicates that the lesions were complete.

In the cerebella of sham-lesioned vehicle control rats the anterior region of cerebellum contained about 150% as much innervation as the other two regions. Neonatal 6-OHDA treatment caused increases in NE, DβH and NE uptake in all regions. The largest increase (60%) occurred in the posterior region while the smallest increase (20 to 30%) was found in the middle region.

In the vehicle control rats the lesion destroyed, in all cerebellar regions, 50 to 60% of the NE innervation of the ipsilateral side and 20 to 30% on the contralateral side. In rats treated neonatally with 6-OHDA the lesion destroyed 75 to 80% of the NE innervation on the ipsilateral side relative to sham-lesioned drug treated levels. No degeneration was detected on the side contralateral to the lesion. These data demonstrate that the locus coeruleus is the source of all 6-OHDA induced sprouting in the cerebellum and this sprouting is exclusively ipsilateral. The normal contralateral projection thus does not regenerate or sprout following the initial neurotoxic effects of 6-OHDA.

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290 DELAYED DISAPPEARANCE OF PLACING AFTER HEMISPHERECTOMY IN THE KITTEN. Joseph E. Bogen, Berry Campbell* and Merton Suzuki*, Ross-Loos Med. Grp., Los Angeles, CA 90026.

After sensorimotor corticectomy, frontal lobectomy or hemispherectomy there is an immediate and enduring loss of paw contact placing in the contralateral limb. A second ablation (of the remaining frontal lobe) results in immediate return of placing (Bogen & Campbell, Science 135:709-710, 1962) suggesting that the previous loss was due to unbalanced, tonic, ipsilateral inhibition. The cortical origin of this inhibition is indicated by subsequent experiments in which hemispherectomy of kittens 10-12 days old is followed by loss of contralateral paw contact placing only after a five week delay. During this delay, chin contact placing and visual placing appear, before subsiding gradually rather than abruptly as in the adult cat. This experiment exposes the ontogenesis of corticofugal influences on a behavior whose essential features appear to be organized subcortically.

291 ELECTROPHYSIOLOGICAL PATTERNS ASSOCIATED WITH NONNUTRITIVE SUCKLING IN 11-13-DAY-OLD RAT PUPS. Stephen C. Brake*, Virginia Wolfson*, and Myron Hofer. Dept. Psychiat., Montefiore Hosp., Bronx, N.Y., 10467.

Previous investigation of suckling patterns in infant rats have employed negative pressure recordings or direct observation of pups. These studies seem to indicate that suckling is a consistent, reflexive response at least over relatively short periods of time. In order to measure more directly activity associated with suckling over time we allowed pups to suckle an anasthetized lactating dam for up to 4 hrs while recording EMG's from the left ventral jaw muscle (digastricus). EMG's were also correlated with simultaneous negative pressure recordings and visual observations. Pups were separated from their mothers either 2-6 hrs or 20-24 hrs prior to testing to assess the effects of differing amounts of maternal deprivation on suckling behavior. At no time was milk available. Muscle activity was scored with respect to overall intensity (amplitude and frequency of EMG) as well as frequency of three different EMG patterns: long rythmic bouts (associated with suction and a clear opening and closing of the mouth), bursting (suction with no visible body movement), and treading (suction and movement of limbs). 20-24 hr pups initially displayed long rythmic bouts which dropped out within 30 min. These bouts were absent in 2-6 hr pups. In both the 20-24 hr and 2-6 hr pups, intensity of muscle tone decreased significantly within an hour. Frequency of bursting and treading remained essentially the same for both groups. Bursting and treading often occurred sequentially, though each occurred independently. 20-24 hr pups, and to a lesser extent 2-6 hr pups, tended to detatch from the nipple after about an hour. These data show specific alteration in patterns of suckling as a function of amount of deprivation and time attached to the nipple. Quantitive analysis of all data will be presented at the meeting.

292 INTERAXONAL CONNECTIONS IN PERIPHERAL NERVES OF NORMAL NEWBORN RATS. G.M. Bray, S. Perkins* and A.J. Aguayo, Division of Neurology, The Montreal General Hospital and McGill University, Montreal, Que.

Vagus nerves , which in newborn rats contain bundles of naked axons, were examined by freeze-fracture and thin-section electron microscopy. On the day of birth, six litters of Sprague-Dawley rats were perfused with 1.5% glutaraldehyde and 0.5% formaldehyde in 0.08 M Sorensen's phosphate buffer. The vagus nerves were removed from each animal and either post-fixed with osmium ferrocyanide and embedded in epoxy resin for thin sectioning or frozen in 20% glycerol, fractured in a Balzers 301 freeze-fracture apparatus, replicated and examined by transmission electron microscopy.

In both longitudinal and cross-sections, many adjacent naked axons were joined by tube-like structures approximately 0.1 μm in diameter and 0.05 μm in length. These structures formed cytoplasmic channels between axons and were surrounded by membranes that were continuous with the adjoining axolemma. These interaxonal communications disappeared as axons became individually ensheathed by Schwann cells.

The presence of large interaxonal connections in newborn rats suggests that axons of mammalian peripheral nerves have a syncytial arrangement during early development. Such connections may be important in metabolic cooperation and other forms of cellular interactions during neurogenesis.

293 QUANTIFICATION OF THE EEG OF THE DEVELOPING RAT[+1]. Bronzino, J.D.[+2], W. B. Forbes, C. A. Tracy*, P. Stisser*, O. Resnick*, and P. J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

In order to quantify the changing patterns seen in the EEG during development of the rat and determine the impact of protein malnutrition on these EEG patterns, we have applied spectral analysis techniques to obtain a "power spectral atlas" of the electroencephalogram of the developing rat. We have analyzed the EEG recorded from several brain sites (specifically, the parietal cortex, frontal cortex, and the hippocampus) in rats that are 90-120 days old. Studies of the power spectra obtained from three other age groups (i.e., 8-14 days, 21-22 days and 30-35 days, postpartum) together with the results from the adult rat group provides a measure of the changing EEG-pattern during development. In the case of the 90-120 day old age group, 42 animals have been implanted with chronic indwelling electrodes and the EEG recordings have been obtained from the 3 brain areas mentioned above during 4 different vigilance states (i.e. waking (W), slow-wave sleep (SWS1, SWS2) and REM sleep). These 42 animals comprised four different dietary treatment groups: (1) 25% protein casein diet, (2) 8% protein casein diet, (3) those reared initially on a 25% diet and switched to 8% at weaning (25-8%), and (4) those reared initially on an 8% diet and switched to 25% at weaning (8-25%). A continuous two hour segment of EEG taken during the same time of day for each animal was scored for the 4 vigilance states and analyzed. This procedure consisted of randomly selecting 8 epochs (8 seconds duration) from each vigilance state, for each brain site (n=3), for each animal (n=42). Using a PDP-11 computer each 8 second epoch of EEG was converted to a digital format using a sampling interval of 7.813 msec., thereby insuring a Nyquist frequency of 64Hz. Fast Fourier Transform routines were then employed to obtain a power spectral representation for each epoch of EEG. The power spectra obtained were then averaged to provide a mean (± standard deviation) power spectrum for that vigilance state. Using this approach, the results obtained from the different age groups and different dietary groups have been compared in a quantitative manner, thus providing a highly sensitive indicator of the EEG of the normally developing rat, as well as, the EEG of the animal subjected to dietary insult during critical periods of its development. The significance of these results will be described in relation to brain ontogeny and the effect of malnutrition on brain development.

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[+2] Also at Trinity College, Hartford, CT

294 BLOOD-BRAIN BARRIER: AGING IN PRIMATES. E. M. Burns and T. Kruckeberg*. Dept. Gen. Nursing, College of Nursing, UIMC, Chicago, IL 60612 and Dept. of Research, Mercy Medical Center, Chicago, IL 60616.

An ultrastructural study of the changes occurring with age in the blood-brain barrier was performed in Macaca nemestrina. Three groups of animals were used in the study: three four-years of age; ten ten-years of age; and seven twenty-years of age, thus representing young, middle-aged and elderly populations. Samples were obtained from the frontal and occipital poles of the cerebrum from all animals for light and electronmicroscopic evaluation of the blood-brain barrier. Samples were also obtained from the posterior pituitary. All samples were fixed by immersion. Full cortical thickness blocks of tissue, not greater than 1mm in width, were postfixed, dehydrated, en bloc stained with uranyl acetate and embedded in epon. Ultrathin sections, perpendicular to the pial surface or perpendicular to the neurohypophyseal surface, were made, further stained on the grid with uranyl acetate and Reynold's lead and photographed either using the Siemens 1A or Phillips 300 electron microscope.

Barrier-type cerebral cortical capillaries were compared with fenestrated neurohypophyseal capillaries in the different age groups. Cerebral cortical and neurohypophyseal capillary profiles were analyzed using the optomax modular television scanning image analysis system (courtesy of μm Micromeasurements Company, Burlington, Mass.). Ultrastructural differences found in the cerebral and neurohypophyseal capillaries of animals from the three age groups will be discussed. (Supported by: #NO1-AG-6-2145 in collaboration with D. M. Bowden, Regional Primate Research Center, Seattle, WA).

295 POSTNATAL MATURATION OF A CHOLINERGIC INFLUENCE ON NEUROLEPTIC CATALEPSY. Dorothy K. Burt, Martha L. Crowner*, Suzanne M. Hungerford*, Katherine L. Melville*, Craig Shanklin* and Luis A. Baez. Dept. Psych. Southern Ill. Univ., Carbondale, Ill.

Catalepsy can be elicited in rats by dopamine receptor blockers and by cholinergic agonists. Neuroleptic-induced catalepsy is antagonized by anticholinergics in adult rats. These and related data suggest that CNS cholinergic and dopaminergic systems interact functionally. This study was designed to investigate the postnatal maturation of this neuropharmacological interaction.

A total of 216 rats were used in the first experiment. Animals of 10, 15 and 20 days of age were injected with saline or one of the following doses of atropine sulfate: 0.156, 0.312, 0.625, 1.25, 5.0 and 20.0 mg/kg. Fifteen minutes later all animals were injected with 1.0 mg/kg spiroperidol, a specific dopamine antagonist. All injections were intraperitoneal. Spiroperidol produced catalepsy at all ages, and this effect was not antagonized by atropine in 10- or 15-day-old animals. At 10 days atropine actually appeared to enhance the cataleptic action of spiroperidol. In 20-day-old rats, on the other hand, catalepsy was reversed by atropine in a powerful and dose-related manner, with 5.0 mg/kg nearly abolishing catalepsy.

A second experiment investigated the cataleptic effects of clozapine. This neuroleptic does not produce catalepsy in adults and has been reported to possess anticholinergic properties in addition to blocking dopamine receptors. Rats of 10, 15 and 20 days of age were injected with saline or clozapine (4.0, 8.0 and 16.0 mg/kg), 12 animals per dose. Clozapine reliably elicited catalepsy in 10- and 15-day-old animals, but failed to do so in the 20-day-old group.

These data suggest that a cholinergic influence on dopaminergic neurons involved in catalepsy becomes functionally mature after fifteen days of age in the rat. Such a hypothesis is consistent with data which indicate that cholinesterase-containing interneurons mature at about this time in the neostriatum (Butcher and Hodge, Brain Res., 1976). However, there appear to be two different cholinergic actions as a function of age. Since there was some indication in the first experiment that atropine might enhance catalepsy in the youngest animals, we tested 10- 15- and 20-day-old animals with one of the following doses of atropine only: 0.156, 0.312, 0.625, 1.25, 5.0, 10.0 and 20.0 mg/kg. Atropine treatment produced slight to moderate catalepsy in 10- and 15-day-olds, but had no cataleptic effect by 20 days of age. Thus, cholinergic blockade has behavioral effects similar to those of dopaminergic blockade up to 15 days of age. After this age, cholinergic blockade antagonizes the effect of dopamine blockade by spiroperidol.

296 REGENERATION OF AN INTERSEGMENTAL INTERNEURON IN THE LEECH. Ronald L. Calabrese. Dept. of Biology, UCSD, La Jolla, CA 92093

The paired heart control neurons of the leech comprise a favorable preparation for studying the regeneration of central synaptic connections, because they are easily identified and penetrated with microelectrodes. The segmental heart motor neurons (HE cells) receive monosynaptic inhibitory input from a set of segmental heart interneurons (HN cells). The HN cell of the third segmental ganglion [cell HN(3)] synaptically contacts ipsilateral HE cells in segmental ganglia 3-18 by an intersegmental axon. Cell HN(3) produces rhythmic impulse bursts at the heartbeat frequency and each ganglionic region of its intersegmental axon can produce these impulse bursts in isolation.

When the intersegmental axon of cell HN(3) is cut and regeneration is prevented, neither the proximal soma + axon nor the distal axonal remnant degenerate even after 18 weeks. Both sections of the severed neuron continue to produce rhythmic impulse bursts and remain connected to appropriate HE cells on their respective sides of the cut. Thus under conditions where regeneration can occur the distal axonal remnant of cell HN(3) presents a possible target for its ingrowing proximal to contact. If such contact were made and through-conduction of impulses restored, then synaptic connection with the HE cells in all segmental ganglia would resume simultaneously. Alternatively, during regeneration the ingrowing cell HN(3) proximal axon could contact the HE cell in each segmental ganglion directly. Resumption of synaptic contact with the various segmental HE cells would thus follow an anterior-posterior temporal sequence and the distal axonal remnant of cell HN(3) would remain to function autonomously.

I have been able to distinguish between these two alternative mechanisms of regeneration by monitoring physiologically the re-establishment of cell HN(3)-HE cell synaptic contacts following severance of the cell HN(3) axon by crushing an interganglionic connective. For 5 weeks following crushes of the cell HN(3) axon no physiological signs of its regeneration were observed in any preparation; both the proximal and distal severed axons remained active and functional but did not conduct impulses across the crush. Regeneration was first observed in some HN(3) cells 6 weeks after crushing their axons; by 9 weeks after the crush the HN(3) cells in all preparations tested had regenerated. The regenerated HN(3) cells always resumed normal synaptic contact with their appropriate HE cells in all segmental ganglia. In such regenerated preparations no signs of a functionally autonomous distal axonal remnant could be detected.

These results indicate that the HN(3) cell regenerates its intersegmental synaptic connections by functionally connecting with its severed distal axonal remnant.

297 RATE OF APPEARANCE OF α-BUNGAROTOXIN BINDING SITES DURING DEVELOPMENT OF CHICK CILIARY GANGLION AND IRIS. V. Chiappinelli* and E. Giacobini. Lab. Neuropsychopharm., Dept. Biobehavioral Sciences, Univ. of Conn., Storrs, CT 06268.

The binding of ^{125}I-α-bungarotoxin (α-BTX) has been examined in the developing chick ciliary ganglion and its end organ, the striated iris muscle. Specific α-BTX binding (Total ^{125}I-α-BTX bound minus ^{125}I-α-BTX bound in the presence of 1000 fold excess unlabelled α-BTX) increases four fold from 7 days of incubation (d.i.) to 11 d.i. (from 4.2 fmole/ganglion to 19 fmole/ganglion), after which the amount of binding remains unchanged up to 4 months after hatching (a.h.), the oldest age tested. Specific binding in the ciliary ganglion is inhibited up to 90% by 10^{-5} M d-tubocurarine, suggesting that, as at the neuromuscular junction, α-BTX binding is associated with the nicotinic cholinergic receptor.

The large increase in binding of α-BTX in the ciliary ganglion occurs after the initiation of ganglionic transmission, similar to the increases seen in choline acetyltransferase (ChAT) and acetylcholinesterase activities (J. Physiol. 257 (1976) 749-766), but while a second period of large increase in ChAT activity begins at 12 d.i., the level of α-BTX binding in the ganglion has already reached its adult value by this time.

In contrast to binding in the ganglion, α-BTX binding in the iris remains very low until 12 d.i., then, soon after functional innervation is established, it increases steadily until 4 months a.h. The total increase from 12 d.i. to 4 months a.h. is almost 40 fold (from 2.8 fmole/iris to 106 fmole/iris).

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298 EFFECT OF AGE ON ODOR ADAPTATION IN HAMSTERS. Catherine Cornwell-Jones. Dept. Psycho., Princeton Univ., Princeton, N.J. 08540

Hamsters 10-56 days old reared in pine shavings prefer the odor of natural pine to the scent of cedar nest shavings in which conspecifics have been housed. Placing pine-reared hamsters in cedar shavings changes their odor preferences, but the particular changes induced depend upon the age of exposure. Following three days of exposure to cedar, cedar nest odor attracts pups 10-12 days old, and is neutral for both juveniles 29-35 days old and for adult males 49-56 days old. Exposed juveniles also acquire a tolerance for the odor of natural cedar shavings which have not housed hamsters, but the odor remains aversive to exposed adult males. The fact that juveniles but not adults adapt to natural cedar odor implies that odor processing systems in the hamster loose some degree of plasticity between puberty and adulthood. Receptor adaptation cannot account for the changes observed in pups or adults. An exposure-induced decrease in receptor sensitivity would have produced indifference rather than the observed attraction to cedar nest odor in pups. The same process would have produced tolerance of both natural cedar and cedar nest odor in adults rather than the observed aversion to the former and tolerance of the latter. The evidence therefore implies that changes in central rather than peripheral mechanisms mediate odor adaptation in hamsters.

299 EMBRYOLOGIC DEVELOPMENT OF A MOUSE SYMPATHETIC GANGLION. Michael D. Coughlin, Dahna M. Boyer* and Ira B. Black. Dept. of Neurology, Cornell University Medical College, N.Y., N.Y. 10021.

Although the postnatal development of the mammalian superior cervical ganglion (SCG) has been studied extensively, regulation of its embryologic development is essentially unknown. The present investigation analyzes the morphological and biochemical ontogeny of embryologic mammalian sympathetic neurons in vivo and in culture.

To define normal development of the SCG in utero, ganglia from litters of different gestational ages were assayed for tyrosine hydroxylase (T-OH) activity, an index of adrenergic neuron maturation. T-OH activity was readily detectable in ganglia of 13-day embryos, and increased approximately 100-fold between this time and birth. Enzyme activity rose gradually between days 13 and 16, increased rapidly between days 16 and 17, and rose gradually once again, between day 17 and birth at 19 days.

Tissue culture studies of the embryonic SCG were undertaken to determine whether embryologic development in vitro paralleled that in vivo, and whether conditions necessary for ganglion differentiation varied during prenatal growth. SCG explants from 13, 14 and 15-day embryos grown in basal medium without added Nerve Growth Factor (NGF) readily adhered to the culture dish surface and exhibited extensive fiber development by 48 hours of incubation. Neurites were maintained for 3-4 days in culture and thereafter began to degenerate. Explants of 14-day gestation ganglia grown in basal medium without added NGF exhibited a marked increase in T-OH activity during the first three days in culture, resulting in more than an 8-fold total rise. This in vitro increase in T-OH activity paralleled that which occurred in vivo: after 3 days in culture T-OH activity was the same as that of the in vivo 16 to 17-day ganglion. Ganglia from 14-day embryos exhibited similar increases in enzyme activity in vitro in the presence of anti-serum to NGF (Anti-NGF) or NGF + Anti-NGF. In direct contrast, ganglia from 17 and 18-day fetuses failed to grow without added NGF, or in medium containing Anti-NGF or NGF + Anti-NGF: virtually no axon outgrowth occurred and T-OH activity decreased by half. These observations suggest that developmental regulatory mechanisms change radically during embryologic and fetal life of the mammalian SCG. (Supported by the Dysautonomia Foundation Inc. and the National Science Foundation.)

300 LONG-LATENCY EVENT-RELATED POTENTIALS IN INFANTS: PROBABILITY-DEPENDENT WAVES. Eric Courchesne, Leo Ganz, Anthony M. Norcia*, and Rachel Y. Courchesne*. Dept. of Psych., Stanford U., Stanford, CA 94305.

Few reports on the averaged event-related potential (ERP) in infants and children have examined waves with latencies later than 250-300 msec. Courchesne (1977) recorded ERPs to tachistoscopically presented slides in 6 to 8 year-old children. He showed that infrequently presented nontargets (stimuli not having experimenter-determined importance) which deviate from an on-going sequence of background stimuli always elicit long latency negative, Nc waves (ca 450 msec) and positive, Pc waves (ca 960 msec) in children; frequently presented nontargets (i.e., backgrounds) do not elicit these Nc and Pc waves. Our study was designed to determine whether or not infants have similar probability-dependent waves. We recorded ERPs from 22 infants, 22 to 32 weeks of age. Slides were flashed for 100 msec onto a screen in front of the infant, and were only presented when the infant was awake, looking at the screen, and not moving. Two sets of slides were used; one set for ten of the infants and the other set for the other ten infants. Each slide in Set 1 contained either an array of 5 red triangles or an identical array of 5 red circles. Each slide in Set 2 contained the face of one of two women; both faces were full-front, eyes open, and occupied the same area on the screen. Infants saw sequences of slides consisting of random orderings of 88% of one member of a slide set and 12% of the other member of a slide set. ERPs to these slides were recorded from Cz, Fz, above the eyebrow, and on the infraorbital ridge; reference was the mastoid.

All stimuli elicited ERPs consisting of high amplitude, long latency negative waves (ca 50 uV at Fz; ca 700 msec) and positive waves (ca 27 uV at Fz; ca 1300 msec); these waves are similar in shape to Nc and Pc waves in 6 to 8 year-olds but are somewhat higher in amplitude and longer in latency. These waves in infants were maximal in amplitude at the same scalp sites as the Nc and Pc waves. As with children, these waves in infants were higher in amplitude to infrequently presented stimuli than to frequently presented stimuli. Given the similarities in amplitude, latency, waveshape, location of maximum amplitude, and sensitivity to stimulus probabilities, it seems that these late negative and positive waves in infants are early representatives of the probability-dependent Nc and Pc waves found in children.

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301 THE SUBCELLULAR DISTRIBUTION OF ENDOGENOUSLY PHOSPHORYLATED PROTEINS FROM CEREBRAL CORTEX OF NEWBORN RATS. Leonard G. Davis* (SPON: Yigal H. Ehrlich). Missouri Institute Psychiatry, Univ. of Missouri-Columbia, Sch. Med., St. Louis, MO 63139.

We have reported recently that specific proteins which serve as endogenous substrates for protein kinase activity in the CNS are unequally distributed among subcellular fractions from the cerebral cortex of mature rats (Ehrlich,Davis,Gilfoil and Brunngraber, Neurochemical Res.,in press). Comparison of the endogenous phosphorylative activity in fractions from cortices of newborn and adult rats may provide information pertaining to the role of cyclic AMP and specific phosphoproteins in the regulation of various neuronal functions. The crude mitochondrial (P2) microsomal (P3) and cytosol (S3) fractions, as well as, preparations enriched in myelin (P2A), synaptosomes (P2B) and mitochondria (P2C) from cerebral cortices of newborn (pups) and adult rats were prepared. Endogenous phosphorylation assays were carried out by incubating aliquots of each fraction with gamma-^{32}P-ATP in the absence and presence of cyclic AMP. The specific proteins which incorporated ^{32}P-phosphate were identified by autoradiography after SDS-slab-gel electrophoresis. The number of phosphorylated bands (16) detected in total homogenates of the pups was the same as in the adult, but the relative distribution of labelled phosphate among the bands differed in the two preparations. The P2 fraction from adult rats demonstrated 12 bands. The phosphorylation of two of these bands (MW=84K,78K) was highly stimulated by cyclic AMP. Corresponding preparations from the pup had low phosphorylative activity toward these two bands and they were not stimulated by cyclic AMP. In addition, bands in the MW range of 15-20K, which in the adult were highly phosphorylated in the absence of cyclic AMP, were minor bands in the pup P2, but they showed cyclic AMP dependent phosphorylation. Two bands (MW= 56K,52K) were more prominent in the pup P2 than in the adult, while another band (47K) was greater in the adult than the pup. The phosphorylation pattern of P3 of pups was similar to that of the adult. The cytosol fractions of adult and pups showed marked differences, particularly, in sensitivity to added cyclic AMP which was higher in the pup. In addition, bands (80K,47K) were major in the pup S3 and minor in the adult S3. The P2A fraction of adult was characterized by high levels of cyclic AMP-independent phosphorylation of bands in the MW range 15-20K which were not detected in the pup. Characteristic bands of osmotically shocked P2B fraction from adult (84K and 78K) were low in the corresponding preparations from pup supporting the suggestion that these bands are involved in synaptic function. Alternatively, a band (47K) of this fraction was more prominent in the pup indicating that this phosphoprotein may have an important function prior to maturation. (Supported by intramural funds from Mo.Inst.Psych.)

302 MATERNAL REGULATION OF SYMPATHETIC NEURON DEVELOPMENT. Mark D. Dibner and Ira B. Black. Dept. of Neurology, Cornell University Medical College, N.Y., N.Y. 10021.

The role of maternal salivary glands in the ontogeny of sympathetic neurons was studied in neonatal rats. Pregnant females underwent bilateral sialectomy on day 11 of gestation, and development of the superior cervical ganglion (SCG) was examined in pups at birth and at various postnatal ages. Salivary gland removal in mothers prevented the normal postnatal development of postsynaptic tyrosine hydroxylase (T-OH) and DOPA decarboxylase activities, and presynaptic choline acetyltransferase activity in the SCG. Total ganglion protein, a measurement of overall SCG growth, also failed to develop normally in progeny of mothers lacking salivary glands. Maternal sialectomy also altered development of other sympathetic ganglia in the offspring: T-OH activity and total protein failed to develop normally in the sixth lumbar sympathetic ganglion. Salivary extirpation in mothers did not affect development of neonatal body weight, suggesting that the effects on sympathetic neuron ontogeny were not caused by nutritional deficits.

Postnatal sialectomy in mothers of 1 and 2 day old, but not 4 or 6 day old animals blocked SCG development to a lesser degree than prenatal maternal sialectomy. These data imply that during fetal and early postnatal life maternal salivary factors regulate sympathetic neuron development.

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303 HIPPOCAMPAL AFTERDISCHARGES AND PRENATAL CARBON MONOXIDE. R.S. Dyer, C.M. Eccles*, S. Swartzwelder*, L.D. Fechter, and Z. Annau. Dept. Environmental Health Sciences, Johns Hopkins Univ., Balto., Md. 21205.

Exposure of pregnant rats to levels of carbon monoxide sufficient to produce a maternal COHb saturation of 15% throughout gestation is known to produce a variety of neurochemical and behavioral changes in neonates. To date, no neurophysiological changes have been shown in neonates subjected to similar exposures, nor have any changes yet been shown using any of these techniques with animals tested as adults. Since there is evidence that neurons in the hippocampal formation are sensitive to hypoxia, and since seizure disorders are among the disturbances reported in cases of perinatal hypoxia, the present investigation was designed to determine the influence of prenatal hypoxia upon adult afterdischarge (AD) properties. Pregnant Long-Evans rats were exposed to either 150 ppm CO or 0 ppm CO throughout gestation. At birth, the exposed females and their litters were removed from the CO, and all litters were reduced to eight. Pups were weaned on post-natal day 21 and housed in groups of 3 or 4 by sex until surgery. On day 65±5 the animals had bipolar nichrome wires implanted into the dorsal hippocampus for stimulation and recording, and skull screws implanted for grounding and cortical recording. Following two weeks recovery, thresholds for producing AD's were determined according to the method described by Racine (EEG, 1972). The results indicated that the first AD elicited from the exposed animals was on the average 30% longer than those elicited from control, but that there was no significant difference between AD thresholds determined for the two groups. There were no apparent sex differences in either threshold or duration of AD. Since longer AD's precede the kindling of motor seizures (Racine, EEG, 1972), it is possible that animals prenatally exposed to low levels of CO kindle more rapidly to motor seizures than controls. This research was supported in part by NIH grants HL 054053 and EHS 00454.

304 GROWTH OF THE OCULAR MOTOR SYSTEM. S. S. Easter, Jr., Institut d'anatomie, Université de Lausanne, 1011 Lausanne, Switzerland, and Division of Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109.

The eye of a goldfish enlarges as the animal grows; the eye diameters of 5-6 and 10-12 cm fish are about 4 and 6 mm, respectively. It is known that the retina grows by adding new neurons (Johns, P. R., Neurosci. Abstr. 2: 1188, 1976). I have examined the ocular motor system to learn how it adjusts to the increased mechanical load of the larger eye.

Individual superior oblique muscles and attached trochlear nerves were dissected from thirteen goldfish 5-12 cm long, which range in size corresponds to a range in age from less than one to between two and three years. Both nerve and muscle were sectioned transversely and examined electron microscopically. Photographic mosaics were made at magnifications of 800X-8000X (muscle) and 2400X-3600X (nerve).

Muscle fibers were identified as membrane-bound profiles containing ordered myofilaments. Fiber diameters ranged from less than 2 to more than 30 um, with the smallest fibers at the outer surface of the muscle. White fibers (those with relatively few mitochondria) were generally larger than red fibers, and the two types were segregated into different parts of the muscle, as noted by Kilarski and Bigaj (Zeit. Zellf. 94: 194, 1969). Cross sectional area of the muscle was larger in the larger animals, due partly to an increase in fiber diameter, but principally to an increase in fiber number, from a mean of 373 (5-6 cm animals) to a mean of 810 (10-12 cm animals). Both red and white fibers became more numerous. This growth by addition of new muscle cells strongly suggests that neuromuscular synaptogenesis continues as the animal grows.

Nerve fibers were all myelinated, with diameters ranging from 2 to 15 um in animals of all sizes. Mean fiber number was slightly higher in the older animals; 81 (5-6 cm animals) vs. 97 (10-12 cm animals), but the variance was too great to permit a firm conclusion as to whether the larger muscles were innervated by more nerve fibers. Some of the variance was due to preterminal branching of the fibers while still in the main trunk, which makes the fiber count dependent on the location at which it was made. (Supported by grant 3.776 from the Swiss National Science Foundation to H. Van der Loos, and by a grant from IBRO-Suisse to S. S. E.)

305 VISUAL EVOKED POTENTIALS IN ADULT RATS PRENATALLY EXPOSED TO METHYLMERCURY. C.M. Eccles* and R.S. Dyer (SPON: Z. Annau). Dept. Environmental Health Sciences, Johns Hopkins Univ., Balto., Md. 21205.

The developing nervous system is known to be particularly sensitive to the toxic effects of methylmercury (MeHg). It has been suggested that visual evoked potential (VEP) recording may be a useful method of detecting altered CNS function in the juvenile offspring of pregnant rats given daily doses of MeHg, (Pharm. Biochem. Behav., 1976, 5, 253). The present experiment tests the possibility that exposure of pregnant rats to a single low dose of MeHg may alter VEP's of offspring tested as adults. Five pregnant rats were given 5 mg/kg MeHg as MeHgCL dissolved in corn oil on day seven of gestation by gastric intubation. Six pregnant control rats received corn oil alone. Litters were reduced to eight on day one and weaned at 22 days of age. No apparent signs of toxicity were seen in the mothers or offspring at any time during gestation or postnatal life. At 60 days of age, skull screws for cortical recording and grounding were implanted in thirteen female offspring born to MeHg-treated mothers and in ten females born to control animals. After a two week recovery period, unanesthetized animals were studied with their pupils dilated in a chamber designed to reflect flashes. During a recording session, an animal's VEP's to 100 light flashes at one of four different intensities were recorded and averaged. Each of the light intensities was presented to each animal twice in counter-balanced order during the course of eight sessions. Peak to peak amplitudes and latencies of the VEP's major components were measured.

The most striking difference was seen in the earliest negative component (P_1-N_1) in which the experimental group showed a 40 percent increase over the control in mean amplitude at all light intensities. The second component, N_1-P_2, of the MeHg animals also showed an increase in mean amplitude for all light intensities but this increase was not statistically significant. Mean latencies of N_1 and P_2 were the same for both groups. It appears that a single low level prenatal exposure to MeHg is sufficient to produce long-term alterations in CNS function, and that the VEP technique is capable of detecting these alterations. This research was supported in part by NIH grants HL 054053 and EHS 00454.

306 EMBRYONIC DEVELOPMENT OF SENSORY NERVES IN AN INSECT. John S. Edwards and Su-Wan Chen* Dept. Zool. Univ. Wash. Seattle, WA 98195

The abdominal cerci of the house cricket Acheta domesticus are an elongate pair of posterior sensory appendages. Their numerous mechanosensory sensilla project to the terminal ganglion, and thence principally to giant interneurons of the ventral nerve cord. Aspects of the structure, function and regeneration of this system have been extensively studied. This report concerns the pattern of embryonic development of sensory axons in the abdominal cerci.

Axon-like processes arising from cells with no discernible sensory apparatus, and presumed to be pioneer fibres appear within the lumen of the cercus toward the end of katatrepsis, that is at about 50% of the full development period. They lie on dorsal and ventral midlines, occupying thus the plane of symmetry for subsequent sensory differentiation. Cells which subsequently wrap afferent axon bundles always accompany the earliest axon-like profiles and may prove to precede them. The first appearance of axons in the presumptive neuropile of the terminal ganglion coincides with that of the pioneer fibres. The population of pioneer fibres increases in number prior to elongation of the cercus and deposition of the first (embryonic) cuticle, on which there are no sensilla. With the deposition of the second embryonic (first instar) cuticle, axons from developing sensilla follow the preformed dorsal and ventral pathways through the cercus and thence to the ganglion via the single cercal sensory nerve during the final 15% development.

The neuropile has an open lacunar structure until the arrival of the massive population of sensory axons.

The presence of axons without apparent sensory function in the instar before that in which functional sensilla develop as described above for the cricket embryo parallels the sequence of events in metamorphosing Lepidoptera. In both cases axon-like processes arising from epidermal cells provide the pathway for subsequent functional axons. Contact between periphery and center is thus achieved when they are in closest proximity.

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307 GOLGI AND EM STUDIES OF POSTNATAL DIFFERENTIATION OF DENDRITIC ARBORS OF INTERNEURONS IN LAYERS II AND III OF TRIGEMINAL NUCLEUS CAUDALIS IN NEWBORN KITTENS. William Falls and Stephen Gobel. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20014.

Most of the axons of primary trigeminal neurons entering the gelatinous band of nucleus caudalis do not synapse directly on second order projection neurons in layer I. They synapse on dendrites of interneurons in layers II and III. It is these interneurons which determine to a great extent what features of the stimulus encoded by the primary neuron will be passed on to the second order projection neuron. Developmental studies were designed to examine the postnatal differentiation of the dendrites and axons of these interneurons. These studies show that the two major interneurons of layers II and III (the stalked cell and the islet cell) are present at birth. The most immature forms of both cell types resemble a sea urchin, with short varicose dendrites radiating out in all directions from their cell bodies. During postnatal differentiation many dendrites bead up and are lost while only a few go on to lengthen. The stalked cell lengthens its medial dendrites and retracts most of its other dendrites while the islet cell lengthens its rostral and caudal dendrites. The lengthening of some dendrites and the retraction of others take place utilizing the same basic cellular mechanism. At many sites within dendrites the agranular reticulum gives rise to numerous vesicles called addition vesicles. These vesicles fuse with the dendritic membrane at numerous sites and become part of it. This sequence is responsible for the lengthening of dendritic membranes as dendrites elongate. In retracting dendrites, addition vesicles, instead of adding to the dendritic membrane, fuse with each other to form small cavities within the dendritic shaft. These cavities continue to enlarge, hollowing out the dendrite. The cavities ultimately become continuous with the intercellular space as their membranes fuse with the dendritic membrane. Finally, retracting dendrites, having been almost completely hollowed out by cavities, fragment and disintegrate.

These studies show that the dendritic arbors of the two major interneurons of layers II and III follow similar developmental sequences. They are laid down in preliminary form prenatally and altered postnatally through selective elongation of some dendrites and retraction of others. Dendritic elongation proceeds not from terminal growth cones but from multiple fusions of addition vesicles along the lengths of dendritic shafts. Finally, addition vesicles are involved in mechanisms underlying dendritic retraction as well as dendritic elongation in both of these cell types.

308 DEFICIENCIES IN THE STRUCTURE OF THE LEECH NERVOUS SYSTEM PRODUCED BY LESIONS TO THE EMBRYONIC NERVE CORD. Juan H. Fernandez* and Gunther S. Stent*. Dept. of Mol. Biol., Univ. California, Berkeley 94720. (SPON: John G. Nicholls).

The ventral nerve cord of the leech originates in the embryo from two rows of stem cells lining the medial surface of its germinal bands. These cells proliferate and differentiate into neuroblasts and glioblasts, which form a longitudinal series of ganglionic primordia. Each ganglion arises by the coalescence of two lateral and one medial primordia lying in register (Fernandez, Neurosc Abstr.; Vol. II, 1976). The stem cells are generated by one pair of neuroteloectoblasts present at the caudal end of the germinal bands. The results of the destruction of the ganglionic primordia and of the neuroteloectoblasts of 6-8 day-old embryos of the leeches Hirudo medicinalis and Haemopis marmorata were studied. The lesions were performed with a fine needle and the embryos were allowed to survive from three days to several weeks after the operation. Destruction of the ganglionic primordia resulted in ganglia deficient in nerve cells. The number and location of the missing nerve cells depended on the extent and location of the lesions. Damage to the medial ganglionic primordia also resulted in development of hemiganglia and formation of split connectives. Destruction of the neuroectoblasts, and of a discrete number of stem cells attached to them, in 6-7 day-old embryos resulted in the formation of ventral nerve cords lacking some of the posterior ganglia. However, the same type of lesions performed in 8 day-old embryos was followed by normal development of all ganglia. These results permit the following conclusions: 1) the possibilities for regulation of the embryonic leech nervous system are limited; and 2) the number of stem cells required to form a complete ventral nerve cord is already present by the 8th day of embryonic development. (Supported by NSF grant BMS 74-24637 and National Foundation-March of Dimes grant 1-499).

309 CELL SURFACE PROTEIN DEVELOPMENT IN POST-MITOTIC, PRE-FUSIONAL CHICK EMBRYO MYOBLASTS, IN VITRO. Barry Festoff and Steven Miller*, Neurobiology Research Lab, V. A. Hospital, Kansas City, MO 64128 and University of Kansas Medical Center

It was previously reported (Festoff, J. Cell Biol. 70:190a, 1976) that cytocholasin B (CB) treatment of chick embryo muscle (CEM) produced (2) populations of cells distinguished by the presence or absence of a large (240,000 daltons) membrane glycoprotein. Fibroblastic cells contained high quantities while myoblasts had little, if any, of this protein. Specific antibody to 3T3 cell surface protein (supplied by Dr. K. Yamada, NIH) decorated fibroblasts and elongated myotubes, but not myoblasts in that study.

The present experiments were performed with mechanically-dissociated, highly-enriched myoblast cultures of 12 day old (Stage 38) CEM. Specific avoidance of trypsin was necessary because of the sensitivity of CSP to proteolytic enzymes. Cells were grown on non-collagen coated cover-slips and harvested at 3, 12, 30, 48, 72 and 96 hrs after plating. After washing 3 times in PBS, cells were incubated successively in goat anti-CSP and then FITC-labeled rabbit anti-goat IgG (Miles Labs) with extensive washing between incubations. In parallel cultures mitotic activity was assessed by ^{3}H Thymidine incorporation and the fusion index was calculated by direct observation. Using a Leitz Orthoplan microscope with BG12, KP480, K510 Ploem filters and incident light, CEM fibroblasts were noted to have diffuse punctate immunofluorescence throughout the course. However, myoblastic elements only developed surface fluorescence after cessation of DNA synthesis but prior to the onset of fusion. All binucleates, myotubes and early myofibers contained large quantities of patchy, solid surface fluorescence. These results suggest that CSP in muscle cells is related to the expression of a particular gene activated after mitotic activity ceases but prior to the onset of fusion. Since CSP has been considered to be an "adhesive" protein (Yamada, K. M. et al., Proc. Nat. Acad. Sci. 73:1217, 1976), its role in muscle cell fusion and morphogenesis requires further study.

310 EFFECT OF PROTEIN MALNUTRITION AT VARIOUS STAGES OF DEVELOPMENT ON SLEEP IN THE RAT. William B. Forbes, Carl A. Tracy*, Peter J. Morgane and Oscar Resnick*. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

In order to elucidate the functional effects of protein malnutrition on the developing brain, we studied sleep behavior in adult rats malnourished at various stages of development. Two isocaloric purified diets containing either 25% (HI) or 8% (LO) casein were used. Mothers of experimental animals were fed one of the two purified diets ad lib. for 5 weeks prior to mating and throughout gestation and lactation. Following weaning at 21 days, pups were either fed the same diet as their mother (groups HI-HI and LO-LO) or switched to the other diet (groups HI-LO and LO-HI). At 70-120 days of age, 8-10 pups from each group were prepared with cortical, hippocampal and neck electrodes. 24-hour polygraphic sleep recordings were collected and visually scored in 12-second epochs using a 3-state classification, i.e. waking (W), slow-wave sleep (SWS) and rapid eye-movement sleep (REM). A 12h:12h light/dark schedule was employed both in the colony room and in the recording chambers.

Malnourished groups (HI-LO, LO-HI, and LO-LO) did not differ from group HI-HI in percent of total recording time spent in W, SWS or REM. In comparison with HI-HI, however, all three malnourished groups exhibited relatively more REM during the dark phase and less during the light phase. REM dark/light ratios (s.e.m.) were as follows: HI-HI = .19 (.03); HI-LO = .33 (.05)*; LO-HI = .40 (.08)*; LO-LO = .45 (.09)*; *differs from HI-HI, $p < .05$, randomization test. Institution of an adequate diet following weaning in group LO-HI did not abolish the effect in adulthood. That the REM redistribution effect was greatest in group LO-LO indicates that the effects of pre- and post-weaning malnutrition are additive, the combination producing a somewhat larger effect than either alone. The reported effect was specific to the REM state, there being no consistent compensatory shift in W or SWS distribution among the malnourished groups. The effect on REM was most pronounced in the early portions of the dark and light phases, just following changes in illumination, suggesting that the effect is due to altered modulation of REM occurrance by illumination rather than to a shift in the underlying circadian rhythm of REM.

These data indicate that maternal dietary protein restriction prior to weaning produces altered sleep behavior in adulthood. Since monoaminergic mechanisms have been implicated in sleep cycle control, these findings may shed light on the nature of the functional impact of previously reported (Stern et al., Exp. Neurol., 1975, 49: 314-326) elevations in brain biogenic amine levels in malnourished rats. Supported by NICHHD grant HD 06364.

311 GENETIC DISSECTION OF THE NUCLEAR-CYTOPLASMIC RELATIONSHIP IN MOSAIC SKELETAL MUSCLE FIBRES. P. M. Frair*, and A. C. Peterson. M. R. C. Group in Developmental Neurobiology, Dept. of Neurosciences, McMaster Univ. Med. Ctr., Hamilton, Ontario, Canada, L8S 4J9.

The multinucleated condition develops in the vertebrate skeletal muscle cell by the fusion of mononucleated myoblasts to form myotubes. In the mature myofibres of mammals the nuclei are located peripherally along the entire length of the cell. Although local membrane specializations occur, most notably at the end-plate region, it is not known if each nucleus controls the biochemical functions of a local territory comprised of the immediately adjacent muscle cytoplasm and membrane, or if gene products of every nucleus are uniformly distributed in the cell. We have approached this problem by measuring the distribution of gene products in single skeletal muscle fibres of adult mouse chimaeras, which contain nuclei of both component genotypes. Adult mouse chimaeras were produced by the aggregation of embryos homozygous for different alleles for the subunit of the cytoplasmic enzyme glucose phosphate isomerase (GPI, EC. 5.3.1.9). Changes in the isozyme composition along the length of a cell would indicate that the enzyme remains localized in cytoplasmic territories. Alternatively, a uniform isozyme profile throughout the cell would suggest that the products of individual nuclei are homogeneously distributed.

We have found that the intracellular distribution of GPI isozymes does not change along the length of single mosaic fibres. In addition, the aggregation of subunits is random. These results indicate that individual myonuclei contribute gene products for the maintenance of the muscle cytoplasm throughout the length of the cell. These observations may provide an insight into the mechanisms of proposed neurotrophic functions.

312 ELECTRON MICROSCOPIC EVIDENCE OF DENDRITE PARTIAL DEAFFERENTATION, LOSS AND STRUCTURAL MODIFICATION IN THE SENESCENT RAT BRAIN. Y. Geinisman, W. Bondareff and J.T. Dodge*. Dept. Anat. Sch. Med., Northwestern Univ., Chicago, IL 60611.

An age-related decrease in total numbers of axo-dendritic synapses per square area was found by us earlier in the supragranular zone of the rat dentate gyrus (Neurosci. Abstr., 1976). This phenomenon could be a consequence of a loss of dendrites, or of a partial deafferentation of dendritic trees involving subsequent changes of dendrite morphology in senescence. The study reported here was undertaken to explore these possibilities.

Tissue blocks containing the rostral portion of the right dentate gyrus were dissected from perfused brains of 5 young adult (3 mo.) and 5 senescent (25 mo.) Fischer-344 rats. A 1 µm thick, methylene blue stained section for measuring the width of the molecular layer and a 750 Å thick, uranyl acetate and lead citrate stained section for obtaining electron micrographs were cut coronally from each rostral and caudal block face. These electron micrographs (20 from each section) were taken from the supragranular zone of the molecular layer in a systematic random manner and were used to study: the number of synapses involving dendritic shafts per tissue square area and per dendrite unit length; the number of dendritic profiles per square area; the dendrite volume fraction and surface area by means of stereological point-counting and intersection-counting procedures.

Comparison of mean group values showed a significant decrease in numbers of synapses involving dendritic shafts per tissue square area (by 35%) and per dendrite unit length (by 40%), in the number of dendritic profiles (by 24%), in the volume fraction (by 12%) and surface area (by 27%) of dendrites for senescent rats relative to young adults. The width of the molecular layer was virtually the same in animals from the two age groups.

These findings suggest: (1) a loss of axo-dendritic synapses and dendritic branches occurs in the supragranular zone of the dentate gyrus of senescent rats; (2) the age-related loss of axo-dendritic synapses is independent, at least in part, of the loss of dendrites because the number of these synapses is decreased per unit length of remaining dendrites in senescent animals; (3) the loss of dendrites involves predominantly smaller dendritic branches, since the volume fraction of dendrites in senescence is decreased less than the number of dendritic profiles; (4) structural modifications of dendrites in senescence include a pronounced decrease in the surface area of dendritic membranes available for synaptic contacts. Therefore, a deficiency of synaptic connectivity seems to be characteristic of the dentate gyrus of senescent rats.

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313 GROWTH AND COLLATERAL SPROUTING OF MESOLIMBIC DOPAMINE NEURONS IN OLFACTORY TUBERCLE OF RAT BRAIN DURING POSTNATAL DEVELOPMENT. G. Gilad and D.J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021.

In adult rats the mesolimbic dopaminergic (DA) neurons of the A10 group innervating the olfactory tubercle (OT) will undergo collateral sprouting in response to denervation of a non-DA input to the OT produced by olfactory bulbectomy (Gilad et al Neurosci Abs 2:813, 1976). We sought: (a) to establish whether DA neurons innervating OT in young rats have the adult capacity to form collateral sprouts, and (b) to relate the sprouting to the postnatal development of the OT and its DA innervation, the former established histologically, the latter biochemically by measuring changes in the activity of tyrosine hydroxylase (TH) and the uptake of 3H-DA. Biochemically the uptake of 3H-DA into synaptosomes of OT at 3 d was 7% of, and at 10 d 58% of the adult levels achieved by 21 d. TH activity reached adult levels more slowly: at birth to d 7 it was 12-15%, it then rapidly rose to reach 75% by 21 d and achieved adult levels by 40 d. The adult pattern of cellular organization, not present at birth, was first seen at 3 d, achieving adult lamination by 7 d. In cell bodies of A10, TH was approximately 45% of adult values at birth, rose to 150% by 14 d and declined to adult values by 21 d. Unilateral olfactory bulbectomy performed in 10 d old pups resulted, 30 d later, in a significant increase, ipsilaterally, in OT of TH to 123% of control and 3H-DA uptake to 137% of control. These changes were comparable to the effects of bulbectomy performed in adults. Olfactory bulbectomy performed in 1 d old pups, killed 40 d later did not produce changes in TH or 3H-DA which differed from unoperated littermates. We conclude: (a) in postnatal development there is a rapid increase of the growth of DA terminals into the OT beginning 7 d after birth, (b) during developmental growth TH activity in the cell bodies is first increased, and then recedes back to adult levels in advance of changes in the development of activity in the OT, (c) the hyperinnervation of OT produced as a consequence of collateral sprouting does not occur in response to deafferentation of the OT of 1 d old, but does so in 10 d old pups. The findings suggest that in mesolimbic DA neurons, dynamic changes in TH activity in cell bodies and terminals are similar in developmental growth and collateral sprouting and that collateral hyperinnervation produced by denervation wil not occur in the OT before a critical developmental period, probably relating to the development of adult organization of the tubercle.

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314 DEVELOPMENT OF MOTOR CORTEX. J. Glass, G. Fromm, and A. Chattha* U. of Pittsburgh, Pittsburgh, PA 15261

The postnatal development of the sensory evoked response has been studied in detail within primary sensory cortex. Of all the sense modalities, the visually evoked response has received the most scrutiny. Studies of slow-wave activity show the response recorded from kitten visual cortex to resemble by the fortieth day of age the response recorded from the adult. The stimulus specificity of single neurons in visual cortex may be present at birth and is certainly fixed by the end of the sixth-eighth week of life. In contrast to these findings from sensory cortex, little work has addressed itself to the timetable for development of the visually evoked response recorded from nonspecific cortex. We have now studied the development of the visually evoked response, slow wave and single units, recorded from a nonspecific area of cat neocortex, the precruciate gyrus "motor cortex".

Visually evoked activity was recorded from motor cortex of chloralose anesthetized kittens at three, four, and five months of age and in adult cats. The slow-wave response from the adult cat is triphasic, negative-positive-negative. At three months of age the response is only a long duration negative wave. At four months of age a secondary positive-negative complex emerges. By the fifth month of life the response is the triphasic negative-positive-negative response similar to the response recorded from the adult.

In the adult, single neurons in motor cortex respond to the light and discharge in synchrony with the occurrence of the positive component of the slow-wave response. In the four month old kittens, the presence or absence of a unit response is correlated with the presence or absence of the positive component. As the microelectrode penetrates the cortex the surface positive component of the slow-wave response becomes isoelectric at 600 µ, reverses its polarity, and reaches a maximum negativity at 1800 µ below the cortical surface.

The laminar analysis of the positive component of the evoked potential and its association with unitary activity suggest that the positive component represents excitatory synaptic activity generated in the deeper layers of the cortex. Our findings therefore indicate a major change during the third through fifth months of life in the sensory-evoked excitatory drive applied to neurons within motor cortex.

315 LONG TERM EFFECTS OF SPINAL TRANSECTION ON THE DEVELOPMENT OF SYMPATHETIC GANGLIA. Robert W. Hamill, Philippe Y. Cochard* and Ira B. Black. Dept. of Neurology, Cornell Univ. Medical College, New York, N.Y. 10021.

The long term effects of interruption of descending central pathways on neuronal maturation was examined in the sixth lumbar (L-6) sympathetic ganglion of the rat. Previous investigations in this laboratory defined the normal maturation of presynaptic choline acetyltransferase (CAT) activity, post-synaptic tyrosine hydroxylase (T-OH) activity and total protein in the L-6 ganglion. Spinal cord transection was performed at the fifth thoracic (T-5) level in 10-11 day old rats and the effect on the long term maturation of the distal L-6 ganglion determined. Spinal transection prevented the normal ontogeny of CAT activity. Enzyme activity was 39% of sham-operated, littermate controls one week and one year after surgery. Similarly, the normal maturation of T-OH activity was prevented by interruption of central pathways, and was 21% of control both one week and one year post-operatively. These observations suggest that descending suprasegmental pathways regulate the normal maturation of intermediolateral column cholinergic neurons and second-order adrenergic neurons in sympathetic ganglia. Additionally, these effects are long lasting as evidenced by the persistent abnormal development one year after surgery. Furthermore, these investigations demonstrate that segmental mechanisms do not allow significant long term recovery of function.

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316 DELETION OF "MISTAKES" IN NERVE-MUSCLE CONNECTIVITY DURING DEVELOPMENT OF RAT EMBRYOES. A.J. Harris* and M.J. Dennis* (SPON: J.H. LaVail). Dept. Physiology, UCSF, San Francisco, CA 94143.

Each internal intercostal muscle in an adult rat is innervated by nerve fibres from the ventral root of the matching thoracic segment. Errors in this segmental arrangement, in which muscle fibres receive innervation from a nerve root of a different spinal segment, are rare. In contrast, intercostal muscles from embryoes up to the 18th day of gestation more frequently (63/167 muscles) contained "mistakes" of this kind. Elimination of "mistaken" connections appears to occur near the time of birth, as they were seen in only 6/120 intercostal muscles from embryoes examined during the period 19 days gestation to 2 days after birth.

Intracellular records from intercostal muscles from 16-18 day embryoes showed that individual muscle fibres were multiply innervated, and that responses to appropriate and inappropriate nerve stimulation could be of equal potency. Multiple innervation from "correct" nerve fibres persisted in 19 and 20 day embryoes, and for some days after birth. Thus deletion of functional connections from inappropriate spinal roots took place earlier than removal of surplus "correct" nerve-muscle connections.

The elimination of inappropriate nerve-muscle synapses during normal development of mammlian embryoes may involve the same mechanisms as the suppression of foreign synapses on adult salamander muscle (Yip and Dennis, 1976).

REF: Yip, J. W. and Dennis, M. J. (1976). Nature, 260, 350-352.

317 EVIDENCE FOR A DUAL REACTION OF NEURONS IN CHICK EMBRYO SPINAL CORD TO EARLY LIMB BUD REMOVAL. Marieta B. Heaton, Dept. Neuroscience, U. of Fl. Coll. Med., Gainesville, Fl., 32610.

The sequence of nerve fiber outgrowth from chick embryo spinal cord was studied following limb bud (LB) removal at day 2 1/2 - 3 of incubation. Previous studies of nerve fiber growth following LB removal (Hamburger, Amer. J. Anat., 102:365, 1958) had implied that in the absence of the peripheral LB tissue, initial growth patterns were aberrant, with fibers growing out immediately to form a neuroma alongside the spinal cord, as if they were immediately responding to a remote message. Viewing the entire sequence of fiber growth, starting shortly after LB removal and continuing for 4-5 days, it was found that initial outgrowth is not altered by the absence of the periphery. Rather, early sensory and motor neuroblasts send a robust complement of fibers to the peripheral-most portion of the embryo available. This observed growth continued for 3 1/2-4 days following the operation, with fiber diameters similar on operated and control sides. Only at day 6 1/2 of incubation did the aberrant growth pattern leading to formation of the neuroma begin to appear. These results indicate that the absence of the periphery is not signalled immediately, as it had previously appeared, but that a dual reaction occurs, consisting of (1) normal fiber outgrowth, followed by (2) abnormal growth leading to neuroma formation. This observation suggests the possibility that some sort of reaction in the cells which send their fibers to the attenuated periphery on the first few days may trigger the response leading to the marked alteration in the normal developmental program of later emerging fibers. Such a reaction could be conceptualized as a transfer of information from the fibers which reach the depleted peripheral area back to their parent cell populations, or an interaction between the cells that die after failing to make peripheral connections and those which have not yet sent out processes.

Another striking consequence of loss of the periphery, during the stages of robust outgrowth, was a severe alteration in fiber elongation, implying a departure in the normal growth rate on the operated side. This disparity was as great as 1:14 in day 7 embryos (fiber length on operated side to that on control side). It is probably significant that the fibers deprived of LB tissue did not exhibit a tendency to wander or seek termination sites. These observations imply that the normal elongation process may depend on some attractive influence exerted by specific peripheral tissue.

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318 AUTORADIOGRAPHIC LOCATION OF [^{125}I]α-BUNGAROTOXIN-BINDING ACTIVITY IN NORMAL AND DEAFFERENTED ANTENNAL LOBES OF MANDUCA SEXTA. John G. Hildebrand and Linda M. Hall. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115 and Dept. Biol., MIT, Cambridge, MA 02139.

Neurochemical and developmental evidence strongly indicates that acetylcholine (ACh) is the principal or exclusive neurotransmitter employed by antennal sensory neurons in the moth Manduca sexta (Devel. Biol. 52: 105, 1976). A major synaptic target of these neurons is the antennal lobe (AL) of the brain. Preliminary anatomical observations suggest that olfactory and possibly other chemosensory neurons project to the glomerular neuropil of the AL, while mechanosensory neurons send their axons to a different neuropil region lying caudo-ventral to the glomerular AL (Walker and Hildebrand, unpublished). Electronmicroscopy has shown that synapses in lepidopterous AL are confined to the glomeruli (Z. Zellforsch. 95: 223, 1969). AL contain an [^{125}I]α-bungarotoxin-binding activity that: (1) is extractable with Triton X-100 and displays saturable toxin binding, which can be blocked by cholinergic drugs; (2) is confined to nervous tissue; and (3) appears gradually during the metamorphic development of the adult brain, even in the absence of antennal afferent input to the developing AL (Brain Res. 119: 389, 1977). In the experiments to be described, we have studied the distribution of the toxin-binding activity in normal developing and mature AL, as well as in AL deprived of their normal afferent inputs. Cryostat sections of Manduca brains that had been lightly fixed with paraformaldehyde were treated with [^{125}I]α-bungarotoxin, washed, and subjected to autoradiography to locate toxin-binding activity in the AL. In normal AL, autoradiographic silver grains are predominantly concentrated over glomeruli in the neuropil, even at early stages of adult development. Binding of labeled toxin to cryostat sections is blocked by pretreatment with cholinergic drugs. In deafferented AL, silver grains are similarly distributed. These abnormal AL, although stunted and ectopic, appear to possess rudimentary glomeruli that bind toxin nearly normally. The density of silver grains over neuronal somata and non-glomerular neuropil of the AL appears to be unaffected by deafferentation. Our findings support the hypotheses that toxin-binding activity is associated with, or identical to, synaptic ACh receptors and that these putative receptors develop essentially normally in quantity and histological location in AL deprived of normal afferent inputs throughout adult development. (Supported by NIH grant NS 11010 and NSF grant BNS 75-22581.)

319 TROCHLEAR NERVE: ONSET OF TRANSMISSION AND DEVELOPMENT OF NEUROMUSCULAR RELATIONSHIPS. R. K. Holt, G. S. Sohal and S. D. Stoney*. Department of Anatomy and Physiology, Medical College of Georgia, Augusta, Georgia 30902.

Development of the trochlear nerve in white Peking ducks was studied to correlate electrophysiological findings with the morphological development of the neuromuscular contacts. Electrophysiological measurements showed that the nerve was functional from day 11 of incubation, the earliest age yet tested, onward. Conduction velocity (at 21-23°C) on day 12 when the nerve is composed of small naked fibers was around 0.2 m/sec, and increased to 5.5 m/sec on day 27 (hatching) and a month after hatching when 95% and 99% of the fibers are myelinated. Stimulation of the nerve with single or multiple current pulses evoked contraction of the superior oblique muscle from day 11 of incubation onward. A progressive increase in the strength of muscle contraction was visually noticed with increase in incubation age.

A modification of Koelle and Friedenwald's stain was used to study the motor endplates and the pattern of innervation (Toop, ST 51:1, 1976). Morphological observations indicate that the trochlear nerve first enters the muscle on day 10 or 11. On day 11 the nerve branches into small fascicles with a few fibers ending directly on myoblasts and myotubes. The first appearance of myofibers is on day 17. Muscle differentiation proceeds from the belly outward and the size of myofibers increases gradually until four weeks after hatching, the latest age yet studied. The motor endplates first become identifiable on day 13 and a majority of them are located in the muscle belly. Most of the endplates on day 13 are innervated by multiple fibers. At hatching virtually all endplates are unineuronally innervated. At intervals of one and four weeks after hatching innervation to the superior oblique muscle is essentially unineuronal.

The results of the present study indicate that: i) trochlear nerve becomes functional as soon as it contacts the muscle; ii) stimulation of the nerve can elicit muscle contraction long before the morphological appearance of the motor endplates; iii) developing superior oblique muscle is multineuronally innervated and becomes unineuronally innervated at hatching; iv) loss of the trochlear neurons and their axons observed during normal development could be related to their failure to establish functional connections with the periphery. (Supported by GRS Grant 5-S01-RR-05365-14)

320 PRODUCTION OF S100 PROTEIN BY CELLS FROM EARLY EMBRYONIC SENSORY GANGLIA IN VITRO. Beatrice Holton* and James A. Weston. Dept. of Biol., Univ. of Oregon, Eugene, OR 97403.

In vertebrate embryos, neural crest cells migrate from the top of the neural tube and differentiate into a variety of phenotypes including melanocytes and elements of the peripheral nervous system. Cultured spinal ganglia from 4-5 day quail embryos contain neurons and a population of small stellate cells, both of which are derived from the neural crest. Stellate cells from spinal ganglia resemble neural crest cells in vitro and can produce melanin pigment under suitable culture conditions. When the stellate cells associate with neurons in vitro, however, they resemble nerve supportive (glial) cells. Large, homogeneous populations of these stellate cells (up to 10^6 cells/35 mm dish) can be obtained in culture. When such cultures are assayed by complement fixation for the presence of S100, a protein localized almost exclusively in nerve supportive tissue, approximately 0.01% of the TCA-precipitable protein is S100. This result provides evidence that this population, in fact, contains differentiated glial cells. The amount of S100 present, however, is at least an order of magnitude lower than levels produced by glial cells in adult animals. Association of glial cells with mature neurons may be necessary for higher S100 production, since high levels of S100 synthesis in vivo appear at the same time as coordinated nerve firing. This suggests that coculture of neurons with cells producing a low level of S100 may induce the latter to produce S100 at a level comparable to that found in mature nervous tissue. Supported by PHS grants CA-16287 and DE-04316.

321 OUTGROWTH OF CUTANEOUS AND PROPRIOCEPTIVE NEURONS FROM CHICK EMBRYO DORSAL ROOT GANGLIA. Marcia G. Honig* (SPON: C. Stevens). Dept. Biol., Yale Univ., New Haven, Conn. 06520.

In the chick embryo, two populations of cells in the dorsal root ganglion (DRG) have been described (Hamburger and Levi-Montalcini, 1949, J. Exp. Zool. 111:457-501) which differ in the time course of their differentiation. The differences are most apparent in 7-15d embryos in which the ventrolateral (VL) region of the DRG contains large cells which differentiate early and the mediodorsal (MD) region smaller cells which differentiate later. It has been suggested, on the basis of the early onset of tactile reflexes (at about 7d) as compared to the later appearance of proprioceptive reflexes (at about 11d), that the VL population consists of cutaneous afferents and the MD population consists of proprioceptive afferents. To test this directly, cutaneous and proprioceptive cell bodies have been localized in lumbosacral ganglia at St 29-32 and at St 36-38 (Hamburger and Hamilton) by exposing the cut ends of nerves to horseradish peroxidase (HRP) or by injecting HRP under the skin or into selected muscles in the hindlimb and allowing retrograde transport to occur.

At early stages the filled cells were always localized predominantly in the VL half of the DRG, in accord with the early birthdays of these cells. At later stages, MD cells as well as VL cells could be labeled. These two patterns of staining were found when either cells sending axons to the skin or cells sending axons to the muscles were filled. Thus neurons growing to different types of peripheral targets have similar distributions in the DRG.

If one assumes that the outgrowth of sensory cell axons is selective so that backfilling cutaneous nerves labels only cells specified to be cutaneous afferents and injecting muscles labels only neurons specified to be proprioceptive, then the following conclusions can be made: 1) Both cutaneous and proprioceptive neurons have sent axons into the limb by St 29; 2) Cutaneous and proprioceptive cell bodies are distributed in the VL half of the ganglion at early stages and throughout the ganglion at later stages; 3) The two cell populations described by Hamburger and Levi-Montalcini do not seem to represent separate classes of cutaneous and proprioceptive cells.

Alternatively, initial outgrowth of both cutaneous and proprioceptive neurons may be non-selective with subsequent death of those that grow to the wrong target; indeed both types of fills often show some degenerating cells which are labeled. This should result in the observed distributed pattern of cell body localization at early stages, with a separation into two distinct populations following the cell death period. However, at St 37, when cell death is essentially complete, a distributed pattern is still seen. Thus, unless there is extensive late migration of cell bodies, the two morphological cell populations do not represent proprioceptive and cutaneous afferents respectively. Further the somas for both type of afferents have similar distributions in the ganglion at all developmental stages.
(Supported by NIH grants NS10666 and NS05768)

322 FINESTRUCTURAL MAPPING OF SYNAPSE FORMATION ON THE SURFACE OF THE MAUTHNER CELL OF THE AXOLOTL (AMBYSTOMA MEXICANUM) J. Jacoby* (SPON: C. Kimmel). Univ. of Oregon, Eugene, Ore. 97403

Electron micrographs were used to map the surface of the Mauthner neuron at 2 stages of the animal's development (40 and 43, which are during the period of rapid dendritic outgrowth). The cell receives a rich supply of morphologically heterogeneous synapses over its surface when mature. The goal of this work is to correlate cell growth and synapse formation.

Maps were prepared which consisted of electron micrographs taken around the surface of the M-cell (identified in sampled thin sections). Plots were made of profiles (synaptic knobs as well as profiles not containing synaptic vesicles) contacting the M-cell surface as well as the associated M-cell inner cell membrane and its adjacent cytoplasm. Two types of measurements were made: 1) the occurrence and distribution over the cell surface of synapses and synaptic knobs; 2) the occurrence and distribution of cytoplasmic cisternae lying on or located near the M-cell inner cell membrane. This was investigated as these structures have been previously suggested as vehicles for the local addition of new membrane during cell growth (Kimmel & Schabtach, J. Comp. Neurol., 156: 49, 1974).

Twelve maps from the stages have been partially analyzed, and a number of patterns have emerged in the distribution of pre- and post-synaptic constituents: 1) Synapses are non-randomly distributed over the cell surface at both stages. 2) The dorsomedial surface of the cell (where the axon emerges from the perikaryon) first receives synapses between these 2 stages, while the dendrites are already innervated at stage 40. 3) On dendrites the number synapses per unit length of membrane increases 50% between 40 and 43. 4) At both stages, cisternae associated with the membrane are slightly more numerous in regions of higher synaptic density. These cisternae also are found more often opposite knobs rather than profiles not containing synaptic vesicles. 5) There is an inverse correlation between the total number of cisternae found locally and the fraction of that total directly on the membrane. This is consistent with the thesis that cisternae are being recruited into the membrane, and that considering also observation 4, the recruitment occurs preferentially near synaptic knobs. This would not necessarily suggest a causal relationship, as there might be some inductive mechanism(s) controlling both events.

323 MORPHOLOGICAL TRANSFORMATIONS OF CELLS AND AXONS IN N. MAGNOCELLULARIS OF DEVELOPING CHICKEN EMBRYOS. Sonal R. Jhaveri* and D. Kent Morest. Department of Anatomy, Harvard Medical School, Boston, MA. 02115, and University of Connecticut, Farmington, CT. 06032.

N. magnocellularis consists predominantly of a homogeneous population of medium-sized cells which receive large, axosomatic endings from the acoustic nerve. The morphological development of these cells and their auditory input was studied using the rapid Golgi and Golgi-Kopsch methods.

At 8-9 days of incubation, cells in n. magnocellularis have round or spindle-shaped perikarya with several long, branched dendrites, which often end in bulbous swellings. Efferent axons have already grown out from the cells, and characteristic terminal plexuses of these axons are seen in n. laminaris bilaterally. By day 10, the dendrites have been replaced by an extravagance of long, somatic processes, giving the cell a very shaggy appearance. This structure is maintained up to day 15, when a remarkable second transformation occurs: the cells lose their processes and present bald, round profiles. Around day 17 or 18, a primitive-looking process, with a tip like a growth cone, emerges from some cell bodies, and somatic spines are evident. By 19-20 days, one or two thin, frail dendritic processes can be seen.

Correlated with this dramatic series of changes in the cells, is a fixed sequence of transformations of the incoming axons. Around day 10, primary sensory axons are seen to end in swellings resembling growth cones. Between days 11 and 13, following the explosive growth of somatic processes, there is a corresponding expansion and ramification of the auditory nerve endings. On day 15, there is a condensation of the terminal axonal branches, which now form a compact, highly branched plexus. In the 16-17 day embryo, the plexus coalesces into a calycine structure now approaching its final form, the end-bulb of Held, which is achieved by days 19-20. The transformation of the plexus to the calycine form occurs around the same time that the cell is denuded of its somatic processes.

A possible causal relationship between the development of the axon and the sequential transformations of the cell is presently under study in embryos with early otocyst removals.

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324 EARLY POSTNATAL SYNAPTOGENESIS IN THE RAT VISUAL CORTEX. Janice M. Juraska and Eva Fifkova. Dept. Psych., Univ. Colo., Boulder, CO 80309.

Although synaptic plasticity in response to environmental events has been clearly demonstrated in the visual cortex of the rat, no detailed data concerning the course of early synaptogenesis in this area are available. In this study, synaptogenesis in the visual cortex of hooded rats at 1, 3, 5, 7 and 10 days of age was examined with electron microscopy. The cortex was divided into the molecular layer, the superficial layers (II-IV) and deep layers (V-VI). In the visual cortex at day 1, very few synapses were present in the molecular and deep layers and virtually none in the yet undifferentiated layers II-IV that compose the cortical plate at this age. The synapses that were present were axodendritic and often symmetrical with little subsynaptic web and few vesicles. There were marked increases in axodendritic synaptic density and maturity with increasing age. Axosomatic synapses were seen as early as day 3 but very rarely. What may have been axospinal synapses first appeared at day 7 and were more frequent by day 10. However, this classification is uncertain since no spinal apparatus was detected. Although the rate of synaptogenesis differed somewhat with layers and age, the largest increases were seen between days 3 and 7, paralleling the dramatic increase in dendritic growth seen at this time in the Golgi picture. By day 10 synaptic density had increased by a factor of 6-7 times in the molecular and deep layers when compared to day 1. The increase was even greater for the superficial layers where almost no synapses were found at day 1. Changes in synaptic length are currently under investigation.

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325 PRENATAL RADIATION EFFECTS ON POSTNATAL DEVELOPMENT OF SQUIRREL MONKEY OFFSPRING. Bernice Kaack and Lary C. Walker* Delta Primate Center, Covington, Louisiana 70433.

Increasing interest has been directed toward the use of the squirrel monkey as a primate model for studying the effects of such environmental hazards as radiation on brain mechanisms, physiology and behavior. Due to the increasing encephalization of functions, extrapolations concerning prenatal effects on sensory, cognitive, motor functions are more likely verifiable in diurnal primates since they are of the same taxonomic order as man. The aims of this study were to examine the effects of 10 and 100R whole-body Cobalt-60 irradiation exposure of the mothers during pregnancy on postnatal development of behavior and physiology of the offspring from birth to 30, 365 and 730 days of postnatal life. Specifically, the offspring were examined for: 1) neuromuscular maturation and sensory-learning-motor behavior; 2) homeostatic regulation of temperature, respiration and heart rate under basal and arousal conditions.

Sham-irradiated control, 10 and 100 R prenatally exposed newborn squirrel monkey infants were removed from their mothers at birth, evaluated for physical anomalies, and raised in a nursery under controlled environmental conditions. Prenatal exposure to 100 rads resulted in a significant impairment during the first 30 days of righting, head-up orientation, tail-hanging reflexes and on climbing performance on an inclined plane. From 30 to 365 days of age, prenatal effects included impairments in maturation of visual acuity and depth perception. There was also a retardation of open field exploration and performance in the Wisconsin General Test Apparatus. Prenatal irradiation resulted in a significant increase in the difference scores between basal and arousal conditions in temperature, respiration and heart rate, particularly during the first 30 days of life. The squirrel monkey females included Columbian, Bolivian and Peruvian subspecies phenotypes. Significant interactions occurred between prenatal irradiation effects on behavior, physiology and phenotypic origin of the offspring. Whereas 10 and 100R dose-response effects were apparent on some behavioral and physiological variables, the sex and phenotypic variance among groups precluded establishment of the 10 rad dose as a threshold dose for subtle effects on behavior and physiology. With inclusion of additional offspring, it will be possible to establish dose-response effects on sensory-learning-motor capacity and on maturation of homeostatic regulation of physiology. (Supported in part by NIH Grant HD09942.)

326 PRIMITIVE SENSORY PATHWAYS ORGANIZE SENSORY LONG TRACTS OF HINDBRAIN AND SPINAL CORD IN XENOPUS TADPOLES. Michael J. Katz* and Raymond J. Lasek. Dept. Anat., Case Western Reserve Univ., Cleve. OH 44106.

A third eye primordium was transplanted to Stage 21-22 Xenopus embryos. The tadpoles were allowed to grow to Stages 46-50 (about 3 weeks) and then the transplanted eyes were injected with tritiated proline. After 3 days the animals were fixed and processed for autoradiographic axon tracing.

Three animals in which eyes developed on the tail and four animals in which eyes developed along the side of the head showed optic axons clearly entering the central nervous system. These transplanted optic nerve fibers followed two tracts, the External Sensory Tract and the Internal Sensory Tract, longitudinally (both rostrally and caudally) regardless of the level of their entry into the nervous system. The tracts were both on the same side of the nervous system and never crossed the midline. They ran from the level of the cerebellum through the hindbrain to the end of the spinal cord. The figure below illustrates the positions of these tracts in representative hindbrain and spinal cord cross sections.

These tracts coincide with the Rohon-Beard cell sensory tracts. In the hindbrain the descending tract of nerve V also follows the Rohon-Beard cell tracts, while in the spinal cord the dorsal root fibers which form the tract of Lissauer will eventually follow the Rohon-Beard cell tracts. (Dorsal root fibers do not appear in the Xenopus spinal cord until after Stage 47.) The transplanted optic axons indicate that the Primitive Sensory Pathways of the Rohon-Beard cells are continuous from the cerebellum to the end of the spinal cord. These long sensory pathways provide the organization for at least three different sensory tracts during development. The existence of Primitive Sensory Pathways suggests the existence of other primitive pathways such as Primitive Motor Pathways.

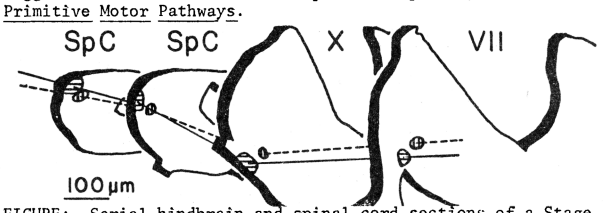

FIGURE: Serial hindbrain and spinal cord sections of a Stage 50 Xenopus tadpole showing two tracts which form the Primitive Sensory Pathways. (VIII=level of entry of nerve VIII, X=level of entry of nerve X, SpC=spinal cord)

327 DEVELOPMENT OF THE SYMPATHETIC INNERVATION TO THE CHICK EYE. Margaret L. Kirby*, Ihsan M. Diab, and Thomas G. Mattio*, Dept. Pharmacological and Physiological Sciences, University of Chicago, Chicago, Illinois, 60637.

Development of parasympathetic innervation of the chick eye occurs over a six-day period including stages 35-40 (8 to 14 days). The nerves include fibers from the ciliary ganglion to the pupillary constrictor, ciliary body and smooth muscle cells in the choroid layer of the eye (Landmesser and Pilar, J. Physiol., 1972). The present study was undertaken to observe the time course of sympathetic innervation of the pupillary dilator using formaldehyde or glyoxylic acid-induced fluorescence for catecholamines. Eyes were removed from chick embryos daily beginning at stage 29 (ca. 6 days) and continuing until 2 weeks posthatching and placed in cold Tyrode's solution. The posterior chamber was opened and the lens, ciliary body and retina were removed. The pigmented layer of the retina and the basal lamina of the choroid coat of the eye were scraped off, leaving the layer of choroid vessels intact. The choroid, including the iris was carefully lifted off of the sclera and cornea, stretched onto a slide, air-dried, and treated to induce fluorescence. At stage 29, the dilatator had a homogenous appearance with a generalized autofluorescent background. During stages 30 and 31, groups of myoepithelial cells and individual capillaries could be seen alternating radially on an autofluorescent background peripheral to the pupillary constrictor. Fluorescent axons could be seen first at stage 39 (13 days) in a circular band just peripheral to the dilatator. Only one or two fibers could be found in the dilatator. By stage 40 (14 days) numerous fluorescent axons with prominent varicosities could be seen in the dilatator proper. At stage 42 (16 days) the innervation of the dilatator was two-thirds complete, but appeared less organized than the innervation of the posthatched iris. In addition to the fluorescent axons in the iris, previously unreported fluorescent ganglion cells could be seen in the choroid proper just peripheral to the iris. These cells first appeared at stage 35 (9 days) as bipolar neurons which then developed into multipolar neurons by stage 39 (13 days). By stage 42 (16 days) the cells contributed to a plexus of fluorescent axons in the choroid. These ganglion cells may contribute sympathetic input to the ciliary body and smooth muscle cells in the choroid, which are known to have parasympathetic innervation. (Supported by PHS 5-PO1 NS-12324-02).

328 LEUCINE INCORPORATION BY IMMATURE AND MATURE AXOTOMIZED FACIAL NEURONS. L. A. Kirschen* and A. LaVelle. Dept. Anat., Coll. Med., Univ. Ill., Chicago, IL 60612.

Differential uptake of (^{3}H) leucine by the facial motor nucleus at selected critical developmental ages and after facial nerve crush at the selected critical ages was examined by liquid scintillation counting. Previous cytomorphological studies have shown that although the hamster facial neurons by 15 days postnatal age have approached the size and morphological configuration of the adult neurons (JEZ 137:285, 1958), their reaction to injury is still not a "mature" one. Chromatolysis occurs without a significant nucleolar change. Only after axotomy at 20 days postnatal age do the facial neurons show the characteristic adult reaction of diffuse chromatolysis accompanied by nucleolar, nuclear and somal swelling. The axon reactions generally peak at 4 days postoperatively.

We used (^{3}H) leucine to examine uptake in hamsters operated on at 15 and 20 days postnatal age and in the adult. The right facial nerves in all animals were crushed, with the opposite sides serving as control. On the 4th day following nerve crush, 2 μCi/gram body weight were injected subcutaneously into each animal. After various post-injection times, the whole facial nuclear groups were removed by dissection, freeze-dried and individually weighed. Unincorporated label was removed, and the tissue was prepared for liquid scintillation counting.

In the 15 day operatives, specific activity was greater on the control sides than on the experimental sides. On the other hand, in the 20 day animals the specific activity was greater on the experimental sides. This latter trend was even further amplified in the adults. These differences between experimental and control sides in all animals were consistent for all post-injection times.

Normal 15 day neuronal groups appear to be operating at maximum synthetic capacity. The additional stress due to injury depresses the already high rate of protein synthesis. The 20 day neurons are more adult-like in that they, as in the adult, are capable of responding to nerve injury by increased protein synthesis. The normal adult neurons seem to be operating well below their protein synthetic capabilities, since injury stimulates the largest increase in protein synthesis. These developmental differences in reaction to injury appear to reflect stage specific levels of metabolism in the hamster facial neurons.

329 DRUG INDUCED TREMOR: EFFECTS OF BRAIN DEVELOPMENT AND ANESTHETICS. W. Douglas Knowles* and M. Ian Phillips (SPON: W.W. Kaelber) Neuroscience Laboratory, Dept. Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52240

Harmaline (10 mg/kg) or tremorine (12 mg/kg) was injected daily into infant rats to determine the age of onset of drug-induced tremor. Tremor to tremorine was recorded in all rats by 8 days of age. Harmaline-induced tremor, however, was not fully established until day 12 in all rats tested. We conclude that these two tremorogenic drugs are acting on different areas of the brain which develop at different ages.

Harmaline has been shown to act on the olivo-cerebellar system (Llinas & Volkind, Exp. Br. Res. 1973) which reaches a mature anatomical structure during the second week after birth (Altman, J. Comp. Neurol. 1972). Electrophysiological recordings were made from the cerebellar cortex of infant rats to determine the age at which the characteristic neural bursting activity appeared in response to harmaline. The development of the bursting activity was found to be coincident with the development of tremor. In all rats by day 12, harmaline induced rhythmic activity of units in the cerebellum. Harmaline provides a useful indicator of cerebellar function and shows that the rat cerebellum is not functionally mature before 12 days of age.

The rate of bursting in the anesthetized infants was approximately one-half the frequency of tremoring in awake infants. The effects of anesthetics on cerebellar function, as measured by harmaline-induced bursting activity, was investigated in unrestrained adult rats using chronic recording techniques. In the awake animal, tremor and bursting occurred at a frequency of 10/sec. Ether (by inhalation) and alcohol (55 ml/kg of 12% ethanol by gastric intubation) blocked bursting activity. Pentobarbital (40 mg/kg, I.P.), chloral hydrate (400 mg/kg, I.P.), and Dial with urethane (0.6 ml/kg, I.P.) slowed the frequency of bursting to 7/sec. and caused bursting to become intermittent. Administration of each anesthetic blocked the tremor.

These experiments demonstrate the use of harmaline and tremorine in the investigation of the functional maturation of the brain and also the functional effects of anesthetics when chronic recordings are made.

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330 LAMINAR DISTRIBUTION OF ACETYLCHOLINESTERASE-DEPENDENT STAINING IN CEREBRAL CORTEX: RELATIONSHIP TO SYNAPTOGENESIS IN NEONATAL RAT. Donald A. Kristt and Darrell V. Lewis*. Dept. Neurol. and Path., Sch. Med., Johns Hopkins Univ., Balto., Md. 21205

Our previous work in neonatal rat has shown that early formed synapses are concentrated in "strata" at specific depths in neocortex, (Br. Res. 108:180(1977). Monoaminergic synapses are found in the strata, but account for only 30% of early synapses, (Neuroscience Lett. 1:305(1975). Neurotransmitter(s) utilized in the majority of early synapses remains unknown. Since acetylcholine is a possible neurotransmitter in adult neocortex, this study was undertaken to determine the pattern of acetylcholinesterase (AchE) dependent staining in postnatal neocortex. Although this technique does not identify cholinergic synapses directly, it does indicate the relative likelihood of cholinergic synapse formation in relation to the cell layers of immature neocortex. Mid-dorsolateral cortex (somatosensory) was studied at birth, P-6 (6 days old) and P-16 with the light and electron microscopes. Staining was achieved by use of an acetylthiocholine histochemical reaction. Staining was blocked by AchE inhibitors. At all ages neocortex stains less intensely than deeper cerebral and brainstem regions. At birth, neocortical staining is barely detectable, despite moderate staining of midbrain and basal forebrain somata and neuropil. By P-6, three heavily stained horizontal bands are seen: a) beneath the pia in the marginal zone (MZ); b) at mid-cortical depths (future layers IV and V); and c) bottom of cortex (future layer VIb). Bands a and b are also regions of high synaptic density at P-6. The MZ shows an interlacing plexus of AchE positive fibers. Occasionally, somata are filled with reaction product (RP). The mid-cortical band, b, exhibits somatic and neuropil staining. Somatic staining is usually restricted to the circular perimeter of the cell body, suggesting cholinergic inputs on these cells. The SI barrel fields for mystacial vibrissae are well stained within band b. Band c in layer VI, mainly is due to intense somatic staining of small non-pyramidal neurons. Cortex at P-16 is similar to P-6, but staining is more intense. Preliminary ultrastructural examination demonstrates RP surrounding many axonal profiles in the 3 AchE positive bands. In the MZ, stained axonal profiles frequently contain 40-50μ circular vesicles. Occasionally RP is seen within a synaptic cleft. In the layer VI band, RP is frequently seen within the Granular ER of small neurons. Based on these findings we speculate that (1) cholinergic cell bodies in layer VI may send axons to form synapses in the marginal zone; (2) extrinsic cholinergic inputs -- perhaps from thalamus -- project to the middle AchE positive band, as suggested by staining of the barrel fields.

331 DEVELOPMENTAL CHANGES IN CEREBRAL PROTEIN TURNOVER. A. Lajtha, D.S. Dunlop*, W. VanElden* and J. Toth*. Research Institute for Neurochemistry, Rockland Research Inst. Ward's Island, New York 10035

Studies of incorporation of amino acids in vitro with various preparations from brain (enriched ribosomal systems, brain slices, homogenates, cell fractions, etc.) showed convincingly that the synthesis of proteins proceeds at a much higher rate in preparations from immature brain than in those from adult brain. In rodents at birth the synthesizing rate is 5- to 10-fold higher than in adult in the period from birth to 10 days. This period corresponds to the rapid deposition of most brain proteins, and its end marks the cessation of cell proliferation. Studies of protein metabolism in vitro, while highly suitable to elucidate aspects such as the mechanisms involved, properties of the systems, and factors influencing metabolism, are not suitable for gaining information about metabolic rates in vivo. We found that protein synthesis in adult brain in vivo occurs at several-fold higher rates than in the "best" in vitro system, while protein breakdown in vivo occurs at several-fold lower rates than in in vitro systems. The developmental changes also can not be compared. Our studies and others, indicate that the stability of immature preparations are greater, and while in young in vitro incorporation is 60-80% that of in vivo, in preparations from adult brain incorporation in vitro is only 5-15% of the in vivo rate. Because of difficulties of measuring the question of whether the metabolism of all proteins or only that of a few are altered during development cannot be answered. We have recently adopted methods to measure cerebral protein metabolism in vivo. Our primary purpose was to keep the specific activity of the administered labeled amino acid constant over a long period of time and to maintain the specific activity of this amino acid in the brain free pool close to that in plasma, to avoid difficulties when the precursor undergoes major changes and to avoid misinterpretations due to compartmentation. For shorter time incorporations (up to 6 hours) the labeled precursor amino acid is administered by constant infusion or in a large flooding dose. For longer time experiments (up to 5 days) (^{14}C)Tyrosine was administered either in oil suspension or as a subcutaneously implanted pellet. Studies on adults showed two major metabolic fractions, a rapidly metabolized one (6 per cent of proteins with an average half-life of 15 h) and a slower pool (94 per cent with a half-life of 10 days). In newborn the rate of synthesis is increased above that needed for growth, resulting in increased breakdown during the most active phase. Long-term incorporation studies indicate that such an increase occurs in a relatively small fraction of the total, and that a major fraction of brain proteins has a turnover in young similar to that in the adult.

332 MORPHOLOGICAL DEVELOPMENT OF ROHON-BEARD CELLS: LOSS OF INTRAMITOCHONDRIAL GRANULES PARALLELS LOSS OF CALCIUM COMPONENT OF THE ACTION POTENTIAL. Janet E. Lamborghini, Marsha Smith*, and Nicholas C. Spitzer. Biology Dept., UCSD, La Jolla, CA 92093.

Three steps may be distinguished in the development of the action potential mechanism in Rohon-Beard cells of Xenopus laevis. At early stages the inward current is carried mainly by Ca^{++} (Nieuwkoop & Faber stages 20-25), later by Ca^{++} and Na^{+} (stages 25-40) and finally by Na^{+} alone (stages 40-51)(Spitzer & Baccaglini, Brain Res. 107:610, 1976). Electrophysiological data indicate that the amount of Ca^{++} entering the cells at early stages should raise the internal calcium concentration by several orders of magnitude and that the calcium is rapidly sequestered (Baccaglini & Spitzer, J. Physiol. in press), perhaps by mitochondria. To examine the morphological consequences of the influx of Ca^{++}, we studied the ultrastructure of the perikaryon of Rohon-Beard cells in stage 22, 30, 37/38, and 42 embryos.

Specimens were fixed in phosphate-buffered formalin-glutaraldehyde with 0.05% $CaCl_2$ and postfixed in phosphate-buffered 1% OsO_4. Rohon-Beard cells were identified by their size and characteristic position in thin sections of whole spinal cord viewed at low magnification. High magnification electron micrographs were taken to reveal detailed mitochondrial morphology. Mitochondrial profiles were counted and scored for the presence or absence of dense intramitochondrial granules. In neurons such granules have been shown by energy dispersive X-ray analysis to contain calcium (Parducz & Joo, JCB 69:513, 1976). Mitochondrial profiles were examined in micrographs from one cell from each of three different specimens in all of the four stages sampled (3 cells/stage): approximately 100 mitochondrial profiles/stage were scored.

In stage 22 Rohon-Beard cells 75±5% (SD, n=3) of the mitochondrial profiles scored contained dense granules; in stage 30, 48±9%; in stage 37/38, 3±3%; and in stage 42, 0%. Therefore, dense intramitochondrial granules, an indication of calcium accumulation in mitochondria, decrease in number in parallel with the loss of the Ca^{++} component of the inward current of the action potential in Rohon-Beard neurons.

The percentage of mitochondrial profiles containing dense granules increased from 48±9% to 63±4% (SD, n=4) in stage 30 animals which were stimulated to swim by longitudinal stroking for five minutes with an eyebrow hair. This increase in stimulated animals is consistent with the proposed role of Rohon-Beard cells as primary sensory neurons which mediate swimming behavior, and suggests that the presence of dense intramitochondrial granules reflects the influx of calcium resulting from electrical activity of the cells.

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333 BRAIN AGING AND PLASMA STEROID LEVELS: QUANTITATIVE CORRELATIONS. Philip W. Landfield and Gary S. Lynch. Dept. Psychobiol., Univ. Calif., Irvine, CA. 92717.

Our previous studies found that the hippocampus of aged rats exhibits a substantial astrogliosis (Landfield et al, J. Gerontol., 1977). Although gliosis is also seen in some fiber tracts and subcortical regions, it is most prominent in the hippocampus and is not seen in other cortical regions. We have also found that the synaptic neurophysiology of the hippocampus is impaired in aged rats (Landfield and Lynch, J. Gerontol.; 1977, Landfield et al, Neuroscience Abstracts, 1976). The hippocampus is known to be a major target region for corticosterone, and is also, in turn, known to influence ACTH secretion. Therefore, we investigated plasma levels of corticosterone and aldosterone in rats of three ages (4 mo., 13 mo., and 25 mo.) and simultaneously quantified the degree of astrogliosis in the hippocampus of each rat. The number of hypertrophied astrocytes increased dramatically as a function of age, with substantial gliosis beginning in the mid-aged (13 mo.) rats. Resting corticosterone and aldosterone levels increased from the young to mid-aged rats and then decreased again in the aged animals. The adrenal glands show a marked hypertrophy in the aged rats, which appears to have just begun in the mid-aged rats. This pattern suggests an initial hyperfunction of the adrenals in mid-age with a subsequent failure of adrenal function in the aged animals.

The most dramatic finding in these studies, however, seems to lie in a significant quantitative correlation that we found between the degree of hippocampal gliosis in individual mid-aged animals and their plasma levels of corticosterone (r=.76). Based upon the known 2-way interactions of the hippocampus and the adrenal system, this finding suggests that steroid levels may be increased because of reduced hippocampal inhibition of ACTH secretion, or that the corticosterone may directly and causally induce an age-related deterioration of hippocampal neurons. If the latter is the case then, at least in rats, the deterioration may begin to accelerate in mid-age. Studies to test these possibilities are underway.

334 THE DEVELOPMENT OF MOTOR PROJECTION PATTERNS IN THE CHICK HINDLIMB. Lynn T. Landmesser. Dept. Biol., Yale Univ., New Haven Conn. 06520.

A previous electrophysiological study (Landmesser and Morris, J. Physiol. 249:301-326) indicated that the pattern of functional synapse formation by chick lumbosacral motoneurons was highly specific from early developmental stages; at day 5 1/2-6 of incubation, each muscle or portion of the primary muscle mass that gives rise to a certain muscle was activated by the same spinal cord segments as the mature muscle. However, erroneous projections, if they were associated with motoneurons that had failed to form effective synapses, would not have been detected. For this reason and to better localize the motoneuron pools contributing to each muscle during development, the present study utilizing the retrograde transport of horseradish peroxidase was undertaken.

Injection of most of the major hindlimb muscles in late embryos (day 10-12) and young hatchlings showed that the motoneurons which project to each muscle form a compact elongate column of cells with a highly reproducible position in both the rostro-caudal and transverse axes (dorso-ventral and medio-lateral) of the cord.

At stage 28 (5 1/2 days), before the major period of normal motoneuron death and individualization of the muscles, (6 1/2-9 days) similar injections of the four primary muscle masses (dorsal thigh, dorsal shank, ventral thigh and ventral shank) were performed. These injections labeled a population of motoneurons whose position in both axes of the cord was essentially similar to that labeled by the injection of the sum of the muscles originating from that mass. Further partial injections within a given muscle mass revealed that before anatomical individualization of muscles, different regions received projections from distinctly different parts of the cord and that these were appropriate for the muscles derived from that region. Similar regional differences were observed in the formation of synapses as revealed by EMG recording of synaptically activated responses elicited by spinal nerve stimulation. This suggests that prior to any major corrections brought about by cell death, motoneurons growing into the limb ramify and make synapses within boundaries that generally conform to their adult projection. Further, the process of muscle individualization that occurs by cleavage of the primary muscle masses does not itself create the specific projection pattern by physically separating axon terminals that exceed their boundaries from their parent somas, resulting in death. (Supported by NIH grant NS 10666)

335 EFFECTS OF EARLY HYPERTHYROIDISM ON THE DISTRIBUTION OF MOSSY FIBERS IN THE RAT HIPPOCAMPUS. Jean M. Lauder and Enrico Mugnaini. Dept. of Biobehavioral Sciences, Univ. of Conn., Storrs, Conn. 06268

Hyperthyroidism, initiated shortly after birth produces well-defined changes in the development of cerebellar granule cells, including the accelerated growth of their axons, the parallel fibers. The present study demonstrates that this hormonal treatment also affects the development of the hippocampal granule cells, resulting in an altered distribution of the mossy fibers.

On the day of birth pups were distributed into litters of 8 males. Animals were injected with a dose of 5-25 µg l-thyroxine from birth until day 2,5,10,15 or 30. Controls were injected with saline during the same period. At 30 days animals were prepared for either the Timm silver sulfide method for staining of mossy fibers, the rapid Golgi method, or electron microscopy.

An ectopic bundle of mossy fibers was consistently formed infrapyramidally in CA3$_{a-b}$ and CA2 at the mid septo-temporal level of the hippocampus as defined by Haug (1974, Fig. 2C). The size of this aberrant layer was clearly dependent on the dose of thyroxine given and the length of administration. The effect was seen with as little as 2 days of hormone injection. Preliminary Golgi and electron microscopic analyses indicate that at least some of these ectopic fibers derive from collaterals of the suprapyramidal mossy fiber bundle, which terminate on "excrescences" located on the basal dendrites of pyramidal cells. Normally infrapyramidal mossy fibers are absent in this region and the basal dendrites do not have excrescences.

The expanded target area of mossy fibers in the hyperthyroid rat indicates that the normal pattern of axonal growth can be *altered experimentally in the hippocampus at a site* where the segregation of afferents appeared so strict as to be paradigmatic. This provides a promising model for the study of synaptogenesis.

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336 EFFECTS OF NEONATAL MEDIAL FOREBRAIN BUNDLE LESIONS ON DEVELOPMENT IN KITTENS. M.S. Levine, C.D. Hull, N.A. Buchwald, L. Erinoff* and A. Heller. MRRC, Sch. of Med., UCLA, Los Angeles, CA 90024. Dept. Pharm. Sch. of Med., Univ. Chic., Chic., Ill 60637.

As part of a series of experiments concerned with the effects of early intervention upon development in the kitten we have been studying the maturation of kittens subjected to bilateral medial forebrain bundle (MFB) lesions at 11-16 days of age. So far, 13 kittens that survived for 46-268 days (mean age at sacrifice 141 days) have had histologically verified lesions of the MFB. Seven of these had large MFB lesions, 6 had smaller lesions. In 9 of these animals the dopamine concentration of the caudate nuclei were analyzed to determine the amount of destruction of the nigrostriatal pathway. Both large and small lesions produced damage to the nigrostriatal pathway evidenced by depletion of dopamine in the caudates (80% and 64% depletion for large [N=3] and small lesions [N=6] respectively). In 6 additional animals the lesions either missed the MFB completely or damaged only a very small portion of it. Dopamine concentrations in the caudates of this group [N=3] approached normal levels although there was some depletion (30%).

Some aspects of behavioral development of the kittens with large and small MFB lesions was compared with that of the kittens that had lesions that missed the MFB and with their intact littermates (N=9). Large MFB lesions produced a transient period of decreased body weight gain (duration 2-10 days). The animals lost weight and had to be hand fed. Kittens with small MFB lesions or lesions in other loci did not display any appreciable period of reduced weight gain. For the first two postnatal months animals with the large MFB lesions typically weighed less than animals in the other three groups. Between 2-3 months of age body-weight of kittens in all groups was approximately the same. *Development of locomotor activity was assessed in an open field.* At about 20-30 days of age the kittens with large MFB lesions displayed more motor activity than kittens in the other groups. This increase in motor activity was sustained and was still apparent at 4-6 months of age. Development of some aspects of learning ability was assessed by determining how well kittens learned a spatial discrimination in order to locate their mother. The results indicated that kittens with large MFB lesions made more initial errors and tended to perseverate in responding incorrectly more often than kittens with small MFB lesions or intact littermates.

Taken together these results indicate that early postnatal damage to the MFB in kittens produces both transient effects (body weight gain) and more persistent behavioral disturbances in motor activity and development of some aspects of learning.

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337 EFFECTS OF NEONATAL AND ADULT TREATMENT WITH 6-HYDROXYDOPAMINE (6-HDA) ON RANDOM-INTERVAL BEHAVIOR. T.E.Levine*, L.Erinoff*, A.Heller and L.S.Seiden, Dept. Pharmacol.&Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Rats were given intraventricular injections of 6-HDA or saline-ascorbate vehicle as neonates (3-days old) and as adults (49,51-days old) according to the following design:

Treatment Group	Treatment as Neonate	Treatment as Adult
Neonatal	6-HDA (100 µg)	Saline-ascorbate
Adult	Saline-ascorbate	6-HDA (2 x 250 µg)
Vehicle	Saline-ascorbate	Saline-ascorbate

At 73 days of age, the animals were trained on a schedule of reinforcement which reinforced responses on the average of once every 90 sec (random interval 90 sec). The animals treated with 6-HDA as adults stabilized at response rates approximately twice those of vehicle control animals, while animals treated with 6-HDA as neonates had response rates which did not differ from those of vehicle control animals. The rates (resp/30 min ± SE) were as follows: neonatal, 736 ± 122; adult, 1228 ± 175; vehicle, 622 ± 88. The animals treated with 6-HDA as adults were much more sensitive to l-Dopa than animals treated with 6-HDA as neonates or vehicle control animals. 25 mg/kg l-Dopa, following peripheral decarboxylase inhibition (Ro 4-4602), decreased rates of adult 6-HDA treated animals to 6% of control. The rates of vehicle-control and neonatal-6-HDA treatment animals were decreased to 40% and 29% of control responding, respectively, by this dose of l-Dopa. There were no differences in the response-rate-decreasing effects of apomorphine (0.006-0.1 mg/kg) among the three groups. The regional brain catecholamine data for the neonatal and adult 6-HDA treated groups are presented as percent of vehicle control in the following table:

	Catecholamine levels in 6-HDA treated animals			
Treatment Group	Striatum (DA)	Telencephalon (NE)	Diencephalon (NE)	Brainstem (NE)
Neonatal	18%	7%	53%	167%
Adult	54%	16%	43%	78%

Although the animals treated with 6-HDA as neonates had greater catecholamine depletions in the striatum and the remainder of telencephalon, they did not show the high rates on the random-interval 90-sec schedule seen in the adult 6-HDA treated animals. These findings suggest that animals treated as neonates are able to compensate for this behavioral effect of catecholamine depletion. (Supported by: USPHS NS-12324-02; RCDA, PHS MH-1056211; PHS 7-F22 ES-02634-02).

338 FINE STRUCTURE OF DEVELOPING CHICK SYMPATHETIC NEURONS, WITH REFERENCE TO STORAGE VESICLES AND GRANULES. L. Luckenbill-Edds and C. Van Horn*. Dept. Neuropathology, Harvard Medical School and Dept. Neuroscience, Mental Retardation Research Ctr., Children's Hospital Medical Ctr., Boston, MA 02115.

We have characterized the fine structure of vesicles and granules that appear during the development of sympathetic neurons in the paravertebral sympathetic ganglia of the chick embryo. Ganglia from the lumbar region of embryonic (E), post-hatching and adult white leghorn chickens were fixed according to Karnovsky's method and with Richardson's permanganate fixative which specifically preserves small dense cored vesicles (sdcv). Ganglia exhibiting aldehyde-induced fluorescence (Grillo et al., 1974) were examined electron microscopically to correlate fluorescence with the presence of large dense cored vesicles (ldcv) and chromaffin-like granules (G).

Adult sympathetic neurons fluoresce a bright green color; they contain many sdcv (40-50 nm) and a few ldcv (100 nm) in processes and in the periphery of cell bodies. When chick sympathetic ganglia first develop (E7), clusters of cells and processes that fluoresce yellow-green and contain predominately ldcv are seen near the capsular region of the ganglion. During the middle of development (E11-13), a more intensely yellow-green fluorescence appears in the same region, correlated with cells having a pleiomorphic population of ldcv and G. In these stages, the granule-containing cells are morphologically distinct from cells identified as developing neurons that have not yet elaborated storage vesicles. By late embryonic (E15-18) and post-hatching stages, few cells fluorescing yellow-green and containing granules are present. At these later stages, sdcv begin to appear in developing neurons, first in the Golgi region, then throughout the perikarya and processes. Concomitantly, the cells assume the bright green fluorescence observed in the adult.

Our results raise questions about the function and fate of the yellow-green fluorescing, granule-containing group of cells found in the embryo, a population of cells that is evidently distinct from sympathetic neurons in its requirements for culturing (Jacobowitz et al., 1975).

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339 HYPOPLASIA OF DAPHNIA OPTIC CENTERS FOLLOWING THE DELETION OF PHOTORECEPTORS AT VARIOUS DEVELOPMENTAL STAGES BY MEANS OF A UV-MICROBEAM. E. R. Macagno. Dept.Bio.Sci.Columbia U,NYC10027.

During a particular developmental stage, photoreceptors of the Daphnia compound eye grow single fibers into the optic lamina, where they sequentially contact a set of undifferentiated neuroblasts. After this contact, which we found to include the formation of transient gap junctions between fibers and neuroblasts and a characteristic wrapping around of neuroblasts on fibers (PNAS 70:433,1973, 71:1099,1974), the laminar neuroblasts begin to differentiate and elaborate their own processes. In order to further explore the role of this interaction in the formation of specific synaptic contacts between photoreceptor and laminar neurons, we have used a UV microbeam to delete small groups of photoreceptors at the following developmental stages: (1)before optic fibers reach the lamina, (2)after they reach the lamina and make gap junctions but before synapses are formed, (3)after synapses begin to appear, and (4)in the adult. The results for stages (1) and (2) indicate a reduced size or hypoplasia of the optic ganglion which arises from both the loss of cells and a loss of processes and hence a reduced neuropil.

Using computer-reconstruction techniques (Fed. Proceed. 33: 2336,1974), we have analyzed the optic ganglion of experimental animals allowed to survive into adulthood in some detail. We have found (a)that the laminar cells whose receptor inputs were completely deleted before or after early contact have degenerated; (b)that, independent of how many photoreceptors are deleted, the ratio of photoreceptors to laminar cells is fairly constant and about the same as the normal value of 8 to 5; (c)that the size reduction is independent of whether the deleted group is lateral or medial and no "empty" spaces are observed; and (d) that a "cascade" effect is seen in that the medulla, a region which follows the lamina, also shows a hypoplasia due to cell loss and a reduced neuropil. The significance of these results, as well as the results for stages (3) and (4), which are now being analyzed, will be discussed in terms of a possible model for the neurodevelopmental process.

340 POSTNATAL MATURATION OF NEURONS IN THE RABBIT VISUAL CORTEX. Patricia E. Marshall* and Lawrence H. Mathers, Jr. (SPON:M.M. Herman). Department of Structural Biology, School of Medicine, Stanford University, Stanford, California 94305

Using the rapid Golgi and Nissl techniques, the postnatal development of the primary visual cortex has been studied. The overall thickness of the cortex is about 750μ at birth, and increases to 1400-1700μ in the adult. Myelination does not begin until the 5th-10th postnatal day and is observed first in the lower layers and the subcortical white matter. At birth there are indications of the cortical cell plate which characterizes fetal cortical development (Mathers, unpublished observations). This structure appears to persist superficial to the developing infragranular layers, but by the 5th postnatal day it is no longer detectable and the adult pattern of cortical lamination has appeared. Also visible in the Golgi preparations of newborn visual cortex are non-neuronal processes extending from the ventricular lumen to the pial surface. These are not impregnated in tissues from animals 5 days postnatal or older.

At birth, pyramidal cells of the infragranular layers are more well-developed than those of the supragranular layers. The basilar dendritic region appears to develop sooner than the apical dendritic region. Spines are impregnated on both dendritic regions from the 5th postnatal day onward. Pyramidal cells appear to be essentially mature by the 10th-15th postnatal day, though the number of dendritic branches increases beyond this time. Stellate cells mature much more slowly, and there appears to be a period of intense growth between the 10th and 20th day. There is also during this period a great increase in the number of dendritic spines found on stellate cells. This sequence of neuronal development corresponds well with physiological data on maturation of receptive field properties in the visual cortex.

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341 NEUROGENESIS IN THE VISUAL SYSTEM OF THE RAT. Lawrence H. Mathers, Jr., Department of Structural Biology, School of Medicine, Stanford University, Stanford, California 94305.

Pregnant Sprague-Dawley rats were injected with ^{3}H-thymidine on the 11th, 14th, 15th, 16th, 17th, 18th, 19th, and 20th day of gestation. The rats were bred in the evening and the next morning was counted as day zero. Each pregnant female was injected with 8uci/gm of body weight. The pups were then delivered and allowed to survive until at least 6 weeks of age. At that time they were anesthetized and perfused with 10% formalin, and paraffin-embedded brains were sectioned at 10u. The sections were coated with Kodak NTB-3 and exposed for 2 months.

In the superior colliculus, labelled neurons were seen in animals who had been injected on the 15th-18th day. In those animals injected on the 15th day, the majority of neurons labelled was in the stratum griseum intermediale (SGI), and in areas deep to this. Very few cells were seen labelled in the superficial gray layers. On the 16th day this pattern persisted, with the exception that some large neurons in the optic layer and the base of the superficial gray were labelled. From the 16th day onward, many neurons in the superficial gray were labelled, and a few in the optic layer and SGI were also labelled.

In the visual cortex, labelled neurons first appeared on the 16th day, in the infragranular layers, and moved progressively upward with each day until at day 19 only neurons at the top of layer 2 and the molecular layer were labelled. In the lateral geniculate, genesis of neurons occurred between days 13 and 15, with a gradient moving from dorsolateral to ventromedial. There did not appear to be a difference in the sizes of neurons labelled at any particular day in the LGN.

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342 PROGRESSIVE LOSS OF GANGLION CELLS IN THE DEVELOPMENT OF CHICK EYES EXPLANTED TO THE CHORIOALLANTOIC MEMBRANE. Steven C. McLoon*, W. Franklin Hughes*, (Spon: W.A.Reynolds). Dept. of Anatomy, Univ. of Ill., and Rush Med. Col., Chicago, Ill., 60612

Early removal of the optic tectum in the chick embryo results in the degeneration of retinal ganglion cells which would ordinarily form connections at this site (Hughes and LaVelle, '75). The present experiments show similar loss of ganglion cells from retinas of eyes allowed to develop on the chorioallantoic membrane (CAM) of host eggs. These eyes develop in the absence of a central target with which to form connections. Explanted eyes were selected at several times prior to the regression of the CAM of the host embryo for study by light and electron microscopy. The eyes, with total development times of 9, 13, and 17 days including the pre-explantation period, were micro-ophthalmic but reasonably well-formed and showed development of retinal photoreceptors comparable to normal eyes of the same age.

At 9 days the retinas of explants are identical in appearance to normal retinas of the same age and show a distinct ganglion cell layer. At 13 days depletion of ganglion cells can be seen in the central retina of explants as compared with normal eyes. Electron micrographs show degenerating ganglion cells and axons interspersed with some apparently normal ganglion cells. By 17 days the degeneration of ganglion cells has progressed to loss of a majority of ganglion cells most notably in the central retina.

These results show that the ganglion cell layer in eyes grown on the CAM undergoes normal histogenesis, but the cells cannot be maintained throughout the developmental period without central connections. The progressive loss of ganglion cells which begins at the center suggests that the onset of degeneration is dependent on the age of the cell since the retina matures in a central to peripheral sequence. It is believed that those cells which do not degenerate may arise from the germinal population at a time later than the majority of cells, and do not degenerate within the time limit imposed by experimental conditions. The results support the idea that there is a critical stage in the development of the ganglion cell after which the formation of central connections is required for the maintenance of the cell.

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343 EFFECT OF MATERNAL DEPRIVATION ON THE PHYSIOLOGICAL AND BEHAVIORAL RESPONSE TO STRESS IN NONHUMAN PRIMATES. G. P. Moberg and W. Mason. University of California, Davis, CA 95616.

The behavioral and physiological response to novel environment and restraint stress was compared in 16 juvenile Rhesus monkeys which had either been raised with mothers or on cloth surrogates. Following removal from the cage, a blood sample was taken for cortisol determination and a telemetry unit for measuring heart rate was attached. The animals were then tested by being (a) returned to the home cage, (b) placed in isolation in an unfamiliar environment, or (c) restrained in a supine position for 10 min. and then placed in a novel environment. During each test period, behavior was observed and upon completion a second blood sample was taken. All testing conditions were sufficiently stressful to result in an increase in cortisol levels; however, there was no significant difference between rearing groups in the adrenal response. Mother raised animals locomoted more while surrogate raised animals tended to show more crouching and self-clasping. While there was no significant difference in heart rates in the home cage, upon being placed in the novel environment, either directly or following restraint, the surrogate raised animals had signicantly lower heart rates. These data indicate that under the conditions studied maternal deprivation had no effect on adrenal cortical response and only minimal effect upon behavior; however, this early experience appears to have influenced the autonomic nervous system's regulation of heart rate.

344 EFFECTS OF DEAFFERENTATION ON DEVELOPMENT OF THE TRIGEMINAL MOTOR NUCLEUS IN CHICK EMBRYOS. S.A.Moody*and M.B.Heaton. (SPON: D. Walker). Dept. Neurosci., Univ. Fla.,Gainesville, FL., 32610.

The theory of neurobiotaxis (Kappers,1917)was formulated in an attempt to account for several basic processes of neurogenesis, i.e. neuroblasts were hypothesized to send processes and to migrate toward those fiber bundles which provided a source of electrical stimulation. Many early studies failed to support the theory. For example, Rhines and Windle (1944) demonstrated that extirpation of forebrain, midbrain or portions of hindbrain in chick embryos had little effect on development of the remaining motor nuclei.

Evidence favoring neurobiotaxis is rare. Levi-Montalcini(1949) found that after unilateral removal of an otocyst all acoustic and vestibular nuclei migrated appropriately, except for the cells of the vestibular nucleus tangentialis. These apparently did not migrate or differentiate and mature on the side deprived of input from the ganglion fibers. Jacobs (1970) suggested that migration of trigeminal motor neuroblasts might similarly be due to stimulation by the sensory root or descending tract fibers. Therefore, we decided to test this hypothesis by removing the trigeminal ganglion primordia and studying resultant migration patterns.

The trigeminal ectodermal placode was removed unilaterally from chick embryos at stage 11-12, and the metencephalic neural crest destroyed by cauterization. These are the primordial regions of the ganglion cells of V (Hamburger and Narayanan,1969). The embryos were allowed to survive until day 9 of incubation.

In all operated embryos the ganglion was missing unilaterally. The remaining ganglion appeared normal and fibers projected several mm into the brain stem. No such central root fibers could be seen on the operated side. In all cases the motor nucleus of the control side consisted of prominent neurons with very large, clear, eccentric nuclei. The cells were either rounded or had one to three processes. These large cells were absent on the operated side in all cases. The area was filled with smaller cells which resembled the cells of the adjacent regions. In a few cases darkly staining, spindle-shaped cells with very dark nuclei were seen. The region deep to the floor of the ventricle seemed to be more densely packed with small, basophilic cells on the operated side.

Thus, the ingrowth of sensory root fibers seems to be essential for the normal development of the trigeminal motor nucleus in the chick. This evidence, in combination with that of the development of nucleus tangentialis, may indicate that sensory root fiber ingrowth is generally a necessary prerequisite to the development of normal brain stem patterns.

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345 LAMINAR ANALYSIS OF ELECTRICALLY EVOKED POTENTIALS IN THE OPTIC TECTUM OF DUCKS DURING DEVELOPMENT. J.B. Munson and M.B. Heaton, Department of Neuroscience, Univ. of Fla. Coll. of Med., Gainesville, Florida

Field potentials were recorded from the optic tectum of duck (Anas platyrhynchos) embryos following electrical stimulation of the contralateral optic nerve head. Recordings were made in embryos on day eleven of incubation through hatching (27th day). Negative potentials were recorded at the dorsal and ventral surfaces of the tectum in all animals. Positive potentials were recorded in the depths of the tectum in all animals. Polarity reversals occurred within 0.5 mm of the respective surfaces. Potentials were reduced or obliterated when stimulus frequencies were increased to 15Hz. In older animals, potentials were more widespread, more complex, larger in amplitude, shorter in latency and of lower threshold than in younger animals. These results are consistent with the generation of postsynaptic activity in the optic tectum of ducks as early as day eleven of incubation. (Supported by EY-01264 and MH-27677)

346 MESSENGER AND NUCLEAR RNA COMPARISONS IN THE NEWBORN AND ADULT MOUSE. Sally Oklund* and William E. Hahn. Dept. Anat. Sch. Med. Univ. Colorado, Denver 80262.

A complex population of messenger RNA is present in the adult mouse brain (Bantle & Hahn, 1976, Cell 8:139; Young et al., 1976, Biochemistry 15:2823). The extent to which alterations occur in nuclear and mRNA populations during postnatal development is not known. We have pursued this problem by hybridizing RNA to labeled unique sequence DNA and cDNA. ^{3}H-cDNA was prepared by transcribing polyadenylated mRNA from total brain of the adult mouse using reverse transcriptase. 90%, or more, of the cDNA was representative of the high abundance class (classes?) of mRNA and the complexity of this DNA was equal to about 1% of the unique sequence fraction of the mouse genome. Hybridization of the cDNA with poly(A) mRNA from adult brain yielded an observed half reaction (=$Cot_{\frac{1}{2}}$) at Cot (concentration x time) 20. A $Cot_{\frac{1}{2}}$ of 60 was observed when mRNA from the newborn was used to drive the reaction. mRNA from the newborn hybridized with the same amount of cDNA as did mRNA from adult brain. These results suggest that essentially all of high abundance class mRNA species present in the adult brain are also present in the newborn. Differences in the observed $Cot_{\frac{1}{2}}$ suggest that the portion of the total mRNA mass comprised by the high abundance class is somewhat lower in the newborn, or that copy frequency of certain species in this class differ markedly between the adult and newborn. The extent to which the cDNA represents "housekeeping" genes which are expressed in all cells, or mRNAs which are "brain specific" is not known. Experiments are in progress to determine whether the complexity of infrequent abundancy classes of mRNA are also similar in the adult and newborn.

Saturation hybridization experiments with unique sequence ^{3}H-DNA show that nuclear RNA from adult, 12-day and newborn are totally overlapping populations of equal complexity. Thus genetic transcription is not grossly altered during postnatal development. Owing to post-transcriptional regulation, this observation does not preclude the possibility of detecting differences in the complexity or composition of the mRNA population which may occur during postnatal development of the brain. (Supported by funds from the NIH and NSF).

347 THE EFFECT OF SPINAL NERVE SECTION ON MOTOR NEURON LOSS DURING DEVELOPMENT IN XENOPUS. Anthony J. Olek*, Charles Edwards. Neurobiology Research Center, Biol. Dept., S.U.N.Y. Albany, Albany, N.Y. 12222

During the development of Xenopus laevis the number of motor neurons in the lumbar ventral horn and the number of axons in the corresponding ventral roots decline. Motor neuron and axon counts are maximal around stage 54, when the limb first shows signs of movement. The counts decrease by approximately 75% during the next few weeks. (Hughes, A. J. Embryol. Exp. Morph. 9:269 1961, Prestige, M.C., Wilson, M.A. J. Embryol. Exp. Morph. 32:819 1974)

The sectioning of one of the spinal nerves innervating the hind limb, before the onset of rapid cell and axon loss, has been found to increase the number of axons in adjacent, untreated nerves, and to increase the number of motor neurons in corresponding spinal cord segments. Either the 10th or 8th spinal nerve was sectioned, the animals were sacrificed two weeks later, and counts were made in the 9th segment of the spinal cord and in ventral root 9. The unoperated, contralateral side was used as a control. The increase in the number of axons in ventral root 9 and of motor neurons in spinal segment 9 ranged from 15-34%. In a few animals sacrificed 4-6 weeks after surgery, the increase in axon numbers appeared to be maintained in ventral root 9 at a time when the number of axons in the treated nerve 8 or 10 approached normal levels. Acute, topical application of solutions of 1.0% colchicine to the spinal nerve produced similar effects to those of nerve section.

This study was aided by a grant from Muscular Dystrophy Association, Inc.

348 AGE DECLINES IN LEARNING, SHORT-TERM MEMORY, AROUSAL AND AGGRESSION IN RELATION TO CELL LOSS FROM THE CORTEX AND HIPPOCAMPUS OF THE RAT. J. M. Ordy and K. R. Brizzee, Delta Primate Center, Covington, Louisiana 70433.

Experiments with human and animal subjects have indicated age declines in learning, short-term memory, arousal and aggression during aging. Cell loss has also been reported from various regions of the cortex and hippocampus during senescence. It has not been established to what extent redundancy of neuronal populations in the cortex and hippocampus is involved in the process of short-term memory consolidation during aging. The aims of this study were to examine life-span changes in behavior with particular emphasis on learning, short-term memory, arousal and pain-elicited aggression in relation to cell loss from the cortex and hippocampus of the Fisher 344 rat. The subjects for this study included 5 young (11 months), 5 adult (17 months) and 5 old (29 months). After behavioral testing, rats were sacrificed for morphometric evaluation of cell populations in the cortex and hippocampus.

The 29 month old group of rats was inferior in passive avoidance learning, 2-6 hour short-term memory retention, arousal and pain-elicited aggression. Although brain weight did not decrease, cortical depth decreased significantly in some regions in old rats. Neuron packing density decreased in visual area 17 but not in auditory area 41. In the hippocampus, cell loss occured in some areas in the old rats. From the results it was concluded that: 1) age declines in short-term memory were related to reduced passive-avoidance learning in the presence of non-significant age declines in other categories of behavior, and 2) age declines in learning and short-term memory of old rats were also correlated with age differences in cell populations not only in the cerebral cortex but in the hippocampus.

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349 THE FUNCTIONAL DEVELOPMENT OF DOPAMINE RECEPTORS IN THE STRIATUM OF THE RAT. D. L. Ormond* and C. Van Hartesveldt (Spon: R. L. Isaacson). Psychology Dept., Univ. of Fla. 32611

Most evidence indicates that the presynaptic terminals of the nigrostriatal dopamine (DA) system in the rat do not mature until at least the fourth postnatal week. However, a DA-sensitive adenylate cyclase was found to be present at birth (Spano et al., 1976) suggesting that the postsynaptic terminals are responsive to DA much sooner than the presynaptic terminals are mature. In the present study, the rotational model of nigrostriatal function was used to assess the functional maturity of striatal DA receptors in the two-day-old rat.

DA (20µg/.5 µl or 10 µg/.25 µl) was injected unilaterally into the neostriatum, olfactory tubercle, or pyriform cortex of two-day-old rats and their postural asymmetry and rotational behavior recorded. Injection of DA at both doses resulted in postural asymmetry contralateral to the side of the injection, as in the adult, whereas injection of saline did not. Contralateral rotation was observed in animals in all three groups receiving DA injections but was most consistently obtained in animals injected into the pyriform cortex. The contralateral asymmetry observed in animals injected into the pyriform cortex and olfactory tubercle could be the result of diffusion of DA back up the cannula track into the striatum. The fact that rotation was observed more in animals injected into the pyriform cortex suggests that this system may be involved in the locomotor component of the rotational response whereas the striatum may be more concerned with the directional component. The results indicate that the DA receptors in the striatum, and probably in the olfactory tubercle and pyriform cortex, are functional in the two-day-old rat.

350 EMBRYONIC DEAFFERENTATION PRODUCES A DELAYED, SECONDARY MIGRATION OF N. ANGULARIS TO AN ECTOPIC POSITION. T.N.Parks and E.W Rubel. Depts. of Otolaryngology and Physiology, Univ. of Virginia Medical School, Charlottesville, VA 22901.

The avian nucleus angularis (NA) is composed of second-order auditory neurons receiving afferent endings from the cochlear nerve. Extirpation of the otocyst in chick embryos at 50-60 hrs. of incubation prevents entry of VIIIth nerve afferents into the brain and produces hypoplastic and hypotrophic development of certain brain stem auditory and vestibular nuclei (Levi-Montalcini, JCN, 91:209, 1949; Parks & Robertson, Anat.Rec. 184:497, 1976). In the present study, the right otocyst was removed at 2.5 days of incubation and at least three embryos were sacrificed at each of the following ages: embryonic days 9,11,13,15,17 and 19 and posthatching day 28. Nissl- and protargol-stained serial sections of the brain or entire head were examined. There was little observable difference in NA between the experimental and control sides until embryonic day 11. At this time, a large group of neurons, which appear to comprise the medial division of NA, can be seen to move ventrally and medially away from their normal position in the extreme dorsolateral aspect of the brain stem. This abnormal migration continues through embryonic day 15 and appears complete by hatching. At this time and subsequently-at least through one month after hatching--the ectopic NA neurons remain in a position ventrolaterad to n. vestibularis lateralis and mediad to the normal position of n. tangentialis, near or within the dorsal part of n. vestibularis descendens. In some cases, a small fascicle of fibers can be seen connecting the ectopic NA neurons to NA pars lateralis, which retains a dorsolateral position. Three lines of evidence suggest that the abnormally-positioned cells are indeed ectopic NA neurons: 1) Their migration can be followed over time from the normal position; 2) Their morphology resembles that of NA neurons; and 3) They do not resemble any neurons in a similar position on the control side or in normal tissue. The abnormal secondary migration of NA begins several days after completion of the normal migrations of surrounding auditory and vestibular neurons and occurs simultaneously with the experimentally-induced atrophic effects observed in NA and n. magnocellularis. Although it appears unlikely that the cochlear nerve influences the initial migration of NA neurons, the finding of a secondary migration to an ectopic position suggests that afferent innervation may serve to stabilize the position of target cells within the brain.

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351 DENDRITIC ATROPHY FOLLOWING COLCHICINE INDUCED NEUROPLASMIC TRANSPORT DISRUPTION: IMPLICATION FOR BRAIN AGING. Ted L. Petit, Dept. Psychol., Univ. Toronto, West Hill, Ont., Canada, M1C 1A4.

A disruption of neuroplasmic transport has been suggested as a possible explanation to account for the dying back of dendritic processes in the aging brain. To test this hypothesis, colchicine, lumicolchicine, or physiological saline was applied in soaked gelfoam pads to the cerebral cortex of the rat. Colchicine is known to disrupt neurotubules and the fast component of neuroplasmic transport. Its isomer, lumicolchicine, which does not bind with neurotubules and has minimal effect on neuroplasmic transport, was used as a control for the non-specific actions of colchicine.

The animals were sacrificed at varying intervals, and their brains subjected to electron microscopic and Golgi analysis. At the electron microscopic level, colchicine application caused a massive disruption of neurotubules. Golgi analysis of colchicine treated cells showed a loss of dendritic spines, followed by dendritic atrophy usually culminating in cell death within 10-15 days. No changes were observed in either lumicolchicine or saline treated brains.

These results clearly indicate that colchicine induced neuroplasmic transport disruption is incompatible with prolonged cell life. Although these dendritic changes bear some resemblance to the condition reported in the human aging brain, colchicine appears to cause a faster and more severe cellular reaction.

352 THE EFFECTS OF VARIOUS PRE AND POSTSYNAPTIC BLOCKING AGENTS ON NATURALLY OCCURRING CELL DEATH IN THE CHICK LUMBAR SPINAL CORD. R. N. Pittman* and R. W. Oppenheim. Neuroembryology Lab., N. C. Dept. of Mental Health, Raleigh, N. C. 27611

Various neuromuscular blocking agents were injected directly into the hindlimb of chick embryos at a time (day 5½-6) when the first neuromuscular contacts are being made. Agents such as α-bungarotoxin, cobrotoxin (Naja naja atra), and α-neurotoxin (Naja naja siamensis) which bind irreversibly to the nicotinic receptors did not affect the time course or extent of naturally occurring cell death. Quantities of d-tubocurarine capable of stopping all motility for the entire 3-day period of cell death produced no apparent effects. Likewise, 4000-6000 LD_{50} (for 20g mouse) of botulinum toxin had no effect on cell death. Therefore, neither an interaction at the receptors nor a release of substances in synaptic vesicles would seem to be essential for motoneuron survival. Work is being continued as to the effects of these agents on endplate formation.

Preliminary work with β-bungarotoxin has shown that this toxin in very small quantities produces a profound increase in the number of motoneurons which die. The possibility that this phenomenon may be due to a mechanism of action other than a phospholipase-A-like activity is currently being investigated.

This system may provide a good in vivo model for studying parameters involved in the initial recognition and early maintenance of neurons.

353 EMERGENCE OF BETWEEN-LIMB SYNCHRONIZATION IN CHICK EMBRYOS. Robert R. Provine, Dept. Psych., Univ. of Md. Balto. Co., Baltimore, MD 21228.

The development of wing and leg movement is described in chick embryos between 7 and 19 days of incubation. (Chicks hatch at 21 days.) The emergence of synchronization between the movements of bilateral limb pairs (the left and right wings and legs) and between an ipsilateral wing and leg was also examined. All active, visually observed limb movements were recorded on an oscillograph which was activated by the experimenter. The records were analyzed in terms of the number of movements occurring during a 10 min. interval and the percentage of total recording time during which motility was present. The amount of between-limb synchronization was interpreted as the duration of concurrent limb motility expressed as a percentage of all limb motility (concurrent and non-concurrent) occurring during a 10 min. interval.

The development of motility in the wings and legs followed a similar course. Motility, expressed either as the number of movements or the percentage of observation time activity was present, increased in all limbs from 7 days up to a peak at about 13 days after which it declined until 19 days. Differences were not detected in the amount of motility of left and right limb pairs. Before 15 days, the wing pairs moved together no more often than a leg pair or an ipsilateral wing and leg. No consistent pattern of between-limb coordination was present. At 15 days and later, there was an increase in within-girdle limb synchronization which appeared concurrently with a decrease in between-girdle synchronization.

In summary, early motility is characterized by considerable independent, jerky limb movement with no obvious patterning existing between the movements of various parts. During development, there is an increase in the proportion of synchronous, in-phase between-wing movements (as in flapping) and 180° out-of-phase leg movements (as in walking). These changes are accompanied by a reduction in concurrent between-girdle movements. These results indicate that there is a dramatic increase in the amount of region-specific spinal cord motor output during the last week of incubation. The present behavioral results do not indicate by themselves whether this transformation is a product of a relative increment in the precision of motor outflow to the limbs or is a shift from unpatterned to patterned motor outflow. (This research supported by NIMH Grant 28476.)

354 CHICK BRAIN CREATINE KINASE ONTOGENY BEFORE THE ADVENT OF CREATINE AND INSULIN. Oscar Ramírez, John Takahashi* and Margarita Hernández*. 831 HSW Cardiovascular Research Institute, University of California Medical Center, San Francisco, CA. 94143 and Departamento de Bioquímica, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México 14, D.F. México.

The ontogeny of brain creatine kinase (CK) was studied during chick embryo development. The cytosolic activity increased 270% in only 10 hours from the 2nd to the 3rd days of incubation; this was followed by a plateau phase throughout development and at the end of incubation another increase of cytosolic (150%) and mitochondrial (374.5%) CK activities occurred from day 17 to day 21 for both sucrose and KCl homogenized tissues. It was observed that 0.1 M-KCl extracted 52% more mitochondrial CK activity than 40 mM-potassium phosphate buffer pH 7.2 when similar samples of mitochondrial fractions were compared at day 21 of incubation. Therefore early embryonic chick brain CK is another "constitutive" enzyme like the early embryonic chick heart CK since creatine has not been enzymatically detected in the embryo until day 4 of incubation. Contrary to previous findings from muscle cultures, insulin does not appear to stimulate the early abrupt increase of brain CK activity since the hormone is not present in the embryo until day 5 of incubation. It is likely that CK increase is associated with neuroblast multiplication at early stages and possibly to neuronal maturation before hatching.

355 ASCENDING, DESCENDING AND TRANSCALLOSAL CONNECTIONS OF THE AUDITORY AND VISUAL FOREBRAIN IN INFANT KITTENS: AN HRP ANALYSIS. Richard Ravizza, Betsy Garlitz* and Paul Cornwell*, Department of Psychology, Pennsylvania State University, University Park, PA 16802.

The thalamocortical, corticotectal, and transcallosal components of the visual and auditory pathways have been studied in three day old kittens using the HRP retrograde transport technique. Over 20 kittens received single (.1 - .3ul) unilateral injections of 40% HRP (Sigma, Type VI) into one of the following structures: inferior colliculus, cortical areas 17-18, 20-21, A1, MSS, or EP. Following a 24 hour survival period each animal was sacrificed, its brain sectioned at 50u, and processed using standard HRP procedures.

Our chief results are threefold. First, numerous conspicuously labeled neurons are invariably seen in dorsal thalamus following each cortical injection. Second, clearly labeled neurons are seen in auditory cortex after injections of the inferior colliculus. Third, labeled neurons are seen in contralateral cortex following cortical injections of HRP.

Taken together these results indicate that these components of the 3 day old kitten sensory pathways are intact shortly after birth. Further, our finding that the thalamocortical and callosal projections are in the vicinity of visual cortex by day 3 stands in contrast to Anker and Cragg's findings based on the Fink-Heimer technique (1). Briefly, they concluded that areas 18 and 19 do not receive thalamic input until days 14 and 21 respectively and do not receive their callosal input until even later. Similar differences in Fink-Heimer and HRP estimates of afferent arrival times at frontal cortex have already been reported by Nauta and Goldman (2). These findings for frontal and visual cortex indicate that for some systems transport techniques may be more sensitive indicators of exactly when axons arrive in the vicinity of their ultimate target.

Anker, R., and Cragg, B. J. Comp. Neuro., 1974, 154, 29-42. Johnson, T., Rosvold, H., Galkin, T., and Goldman, P., J. Comp. Neuro., 1976, 166, 427-444.

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356 BEHAVIORAL AND BIOCHEMICAL ANALYSIS OF GAMMA-AMINOBUTYRIC ACID MEDIATED INHIBITION IN THE EARLY CHICK EMBRYO. J. Reitzel*, R. W. Oppenheim and J. L. Maderdrut* (SPON: I-Wu Chu-Wang). Res. Div. of Mental Health Serv., Raleigh, N. C. 27611; Neurobiology Curriculum, U.N.C. Medical School, Chapel Hill, N. C. 27514.

Effects of exogenously administered gamma-aminobutyric acid (GABA), bicuculline and/or picrotoxin upon spontaneous motility of the chick embryo were observed at 4, 6, 7 and 9 days of incubation. GABA, an inhibitory neurotransmitter, was found to decrease motility at each age, whereas the GABA blocking agents bicuculline and picrotoxin produced the expected motility increases at each age studied. Antagonism of the GABA produced motility decrease was observed when GABA and picrotoxin were administered simultaneously.

Spinal cords from chicks 3, 4, 5, 6 and 7 days of incubation were examined for glutamate decarboxylase (GAD) activity using a radiometric cation exchange method. GAD is considered a marker for GABA secreting synaptic terminals in the adult animals. Significant GAD activity was found at all ages studied. GABA transaminase, the most important degradative enzyme of GABA,was found to be present at least as early as 5 days of incubation.

Although synaptogenesis is just beginning in the spinal cord at these stages, these findings suggest that GABA secreting synapses are present and behaviorally functional from 4 days of incubation onward.

357 PERIPHERAL NERVE REGENERATION IN THE STUMP TAIL MONKEY: EFFECTS OF TRIAMCINOLONE ACETONIDE TREATMENT. Irena Rusenas, William P. Graham, III and Stephen H. Miller. Dept. of Surg., M.S. Hershey Med. Cent. of the Penn. State Univ., Hershey, PA 17033

The efficacy of triamcinolone acetonide (Kenalog) in enhancing nerve regeneration was studied in 20 stump tail monkeys (M. arctoides). After a clean surgical transection of the median nerve proximal to the wrist (n=40) and the posterior tibial nerve above the malleolus (n=40), the nerves were immediately repaired using microsurgical technique and epineural suturing. On a double blind basis, either triamcinolone or carrier vehicle without the active steroid was instilled about the site of the nerve repair after wound closure. Myocutaneous nerve conduction velocity and latency determinations were made at monthly intervals for 18 mos. The neurorrhaphies were removed at 12, 14, 18 and 18 months and processed for light and electron microscopic observation.

Percutaneous stimulation of the repaired nerves yielded an evoked electromyographic response from recording needle electrodes in the thenar musculature at three months and at four months from the posterior tibial nerve. Although the amplitudes were markedly reduced from control levels and the latencies prolonged, a distinction could be made between the two groups on the basis of a shorter time course of the fibrillatory response following the initial evoked peak. This indicated to us even in the early regenerative period, a larger number of innervated muscle fibers in one group. Eight months following repair of the median nerve, latencies and conduction velocities were within normal limits in contrast to the placebo group which advanced to this status at 14 months. This data indicate to us a more rapid attainment of appropriate fiber diameter in those nerves treated with triamcinolone.

Examination of osmicated cross sections of nerves distal to the repair supported the electrophysiological results. In the placebo treated nerves, several fascicles contained very few tiny myelinated fibers whereas no areas devoid of large myelinated fibers were seen in the triamcinolone treated preparations. In general, the fibers in the triamcinolone treated nerves were comparable to controls in size and density, while those treated with vehicle were smaller and less numerous. Longitudinal sections through the area of the repair show less connective tissue proliferation, less dispersal of axons into the connective tissue and a marked reduction in the size of the scar tissue at the site of the repair in those nerves treated with steroid.

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358 EXCESSIVE POLYNEURONAL INNERVATION OF PARASYMPATHETIC NEURONS UPON REINNERVATION FOLLOWING NERVE CRUSH. P. B. Sargent and M. J. Dennis*. Depts. of Physiology and of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

The degree of polyneuronal innervation of frog cardiac parasympathetic ganglion cells was measured in normal animals and at various times following vagus nerve crush. The number of inputs impinging on each cell was determined by recording intracellularly from ganglion cells while independently stimulating each of the two cardiac branches of the vagosympathetic nerves. Normally, about equal numbers of ganglion cells receive one and two synaptic inputs, and few cells (8%) receive more than two inputs. When the vagosympathetic nerves are crushed, synaptic transmission fails in 2-4 days and the ganglion cells remain denervated for 3 weeks. During the ensuing 5 weeks synapses are reformed at a rate of 0.4 inputs per cell per week. By the end of this period, when 93% of the cells have been reinnervated, the pattern of multiple innervation is different than normal: two-thirds of the cells are multiply innervated and 31% have at least 3 synaptic inputs. This pattern remains essentially unchanged for another year. In ganglia examined 14 months after nerve crush significantly more cells than normal were multiply innervated (78%) and significantly more cells received at least 3 synaptic inputs (35%). These findings were corroborated by histological results which showed that reinnervated ganglion cells often received synaptic terminals from two or more axons. We conclude that polyneuronal innervation of ganglion cells is more widespread after reinnervation than it is normally. Thus, whatever mechanisms may have acted during development to limit the extent of polyneuronal innervation appear to be absent during adult life. (Supported by USPHS Grants NS00303 and NS10792.)

359 DEVELOPMENT OF NEURONS AND SYNAPSES IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. S. Schacher* and E. Kandel. Div. Neurobiol. & Behav., Dept. Physiol., Columbia P&S, New York, N.Y. 10032.

It is now possible to grow the gastropod mollusk Aplysia californica in the laboratory from fertilization to adult. In addition, the phenotypic stages of the developing animal and the sequential development of the ganglia comprising its nervous system have been described (Kriegstein, 1977a;b). This provided the groundwork for investigating the developmental mechanisms by which individual identifiable neurons in the abdominal ganglion acquire their characteristic physiological, biochemical and morphological properties.

The present study deals with the early pre-metamorphic development of the abdominal ganglion (16-35 days post-hatching). Initially (16-17 days) each hemiganglion consists of a small group of cells (4-6) surrounding a neuropil containing predominantly incoming fibers from the pleuro-abdominal connectives. During the next 18 days cell number in the hemiganglia increases to 90 neurons and the average cell body volume increases from 25 μm^3 to 65 μm^3. During this time the neuropil also displays an increase in complexity. Differentiating neurons send out primary processes, and in some instances secondary branching, into the neuropil. Even at the earliest stages, morphologically identifiable synaptic contacts are observed. Three classes of postsynaptic elements are found: 1) small spines, 2) larger processes, and 3) cell bodies. Since the axo-somatic contacts are absent in later developmental stages and adults, they may represent initial contacts on individual cells that may later 'move' on to more appropriate contact sites or they may be transient contacts that are later retracted.

An interesting feature of the developing nervous system is that individual ganglia are embedded within a group of 'support' cells containing large secretory granules, 1 to 5 µm in diameter. Following metamorphosis, the granules disappear coincident with an acceleration in growth of the neurons. To examine the possible causal relationship between granule release and neuronal growth, we have released the granules prematurely at early developmental stages by exposing animals briefly to artificial sea water containing high K^+ and low Ca^{++}. Under these conditions, other secretory cells as well as synapses appear to be unaffected. We are currently studying the effects of granule release by comparing the developmental parameters described above in treated animals with those of normal untreated animals. These comparisons may permit us to determine whether the granules have a specific role in the growth and differentiation of neurons in Aplysia.

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360 PLASTICITY IN THE SENESCENT RAT: ANALYSIS OF AXON SPROUTING IN THE DENTATE GYRUS. Stephen W. Scheff, Larry S. Benardo* and Carl W. Cotman. Dept. of Psychobiology, Univ. Calif., Irvine, CA 92717.

It is well known that neuronal loss occurs with age in the brain. However, it is not known whether or not cell loss results in axon sprouting and reorganization of existing circuitry. An understanding of the reorganizational capacity of the aged brain might provide insights into the basic mechanism underlying changes in mental capacity. The present study was undertaken to investigate the capacity of senescent brain to support axon sprouting following partial denervation.

The dentate gyrus of the hippocampal formation was used as a model system and the response of afferents following unilateral lesions studied in 3 month old and 24 month old Fischer 344 rats. In young animals removal of unilateral entorhinal input to granule cells elicits reorganization actualized by an outgrowth of the commissural-associational fiber plexus, increased positive staining of AChE, and a repopulation of the dendritic field as seen by ultrastructural analysis. By each measure there is very little variability between animals. Aged animals respond to such a lesion with similar circuitry changes but less dramatically. The outgrowth of the commissural-associational fiber plexus is on the average reduced, AChE positive staining is less pronounced and the dendritic field does not appear to repopulate to the same degree as in younger animals. Although axon sprouting does occur, a response to specific denervation is typified by a wide range. While some animals show robust capabilities of plasticity like the younger animals other show almost none.

Our findings are particularly interesting in that they correlate with other findings from behavior studies which also indicate that old animals are a nonuniform population, showing variable performance. It may be that inherent plasticity properties of senescent nervous system correlate with the decline in mental function. (Supported by research grant AG 00538).

361 THE DEVELOPMENT OF CATECHOLAMINE FIBERS IN THE PRENATAL CEREBRAL CORTEX OF THE RAT. M. Schlumpf, W. J. Shoemaker and F. E. Bloom. Pharmakol. Inst., Univ. Zurich, Switzerland and The Salk Inst., La Jolla, CA 92037.

During the last gestational week, the cerebral cortex of the rat is progressively innervated by catecholamine (CA) fibers in a rostro-caudal gradient. On gestational day 16, the first CA fibers reach the surface of the lateral part of the developing cerebral cortex. The innervation occurs in a characteristic bifurcated pattern, similar to the pattern of synapse formation in the prenatal rat cortex. Comparison of sections prepared for fluorescence histology or Nissl stain suggests that the superficial CA fibers are within the marginal layer, while the second, and deeper, CA fiber band is located below the developing cortical plate near the intermediate zone. The varicose fibers within the marginal layer are horizontally directed and initially consist of a single layered band of fibers which develops into a 2-3 layered band. The deeper CA fibers around the intermediate zone appear as a thicker layer exhibiting diffusely arranged fibers. During prenatal development, both CA fiber bands extend over a progressively larger part of the neocortical rim in a fronto-caudal direction. The zone between superficial and deeper CA fibers is filled by vertically-oriented neuroblastic cells. This zone is crossed by CA fibers very rarely and only in the later developmental stages. After gestational day 17, another strong CA fiber input is observed from the midline into the prospective anterior cingulate cortex areas. The developing CA fiber system is highly organized: these fibers tend to aggregate around ontogenetically and phylogenetically older structures of the cerebral cortex and are generally not associated with neuroblastic cells. Preliminary neurochemical observations on embryonic rat cortex suggest that norepinephrine is the predominant catecholamine present. We conclude that CA fibers in the cerebral cortex of the rat develop early in the third gestational week, a short time after differentiation of the CA synthesizing cell groups of the brain stem. The functional significance of this prenatal innervation remains obscure. That cortical CA terminals make synaptic contacts by postnatal day 3 has been reported, but the permanence of these synapses has not been established.

362 STUDIES ON THE CYCLIC AMP SYSTEM IN HUMAN BRAIN DURING AGING. M. J. Schmidt* and B. Ghetti* (SPON: I. Slater). Lilly Research Labs. and Indiana University Medical Center, Indianapolis, IN 46206.

Cyclic AMP (cAMP) and cyclic GMP (cGMP) concentrations, norepinephrine-stimulated accumulation of cAMP in tissue slices, and cyclic AMP-dependent protein kinase activity were measured in the cerebral cortex and cerebellum of humans 6-12 hours after death. Similar determinations were made on rat cortex 10 min - 16 hrs after death to assess the effects of post-mortem decay on these systems.

Concentrations of cyclic nucleotides in rat brain declined markedly between 10 min and 6 hrs after death, but there was little further decrease during the next 10 hrs. Cyclic GMP was not detected in the cerebral cortex of rats that had been dead longer than 6 hours, and there was a significant loss of cGMP in the cerebellum at this time. Norepinephrine stimulated the accumulation of cAMP in cortical slices from rat brain at all times after death. During the post-mortem period there was a slight decline in the activity of protein kinase assayed in the presence of cAMP and an elevation in activity assayed in the absence of cAMP. Thus, the percentage stimulation of protein kinase by 10^{-6}M cAMP was somewhat greater 16 hrs after death.

These studies demonstrated that valid estimates of cAMP concentrations, protein kinase activity, and hormone-stimulated cAMP accumulation could be made on brain tissues obtained at autopsy. Cyclic GMP appears to decrease rapidly after death and so was not examined in human material.

Cyclic AMP was found in the cerebral cortex and cerebellum of humans although concentrations were lower than those in rat cortex. Levels were comparable in 2-3 day-old infants and 60-80 year-old patients. Norepinephrine stimulated cAMP accumulation only in the cerebral cortex of very young humans. The maximal increase occurred between 5 and 10 min of incubation with 10^{-5}M norepinephrine. No elevation of cAMP was seen in the cerebellum at any age or any concentration of norepinephrine. Protein kinase was found in the cerebral cortex of humans as early as 2 days after birth. The enzyme was maximally activated by 10^{-6}M cAMP. There was no change in activity with increasing age. Kinase activity in human cerebral cortex was comparable to that in samples of rat cerebral cortex.

363 REGIONAL DIFFERENCES IN CRITICAL PERIODS FOR 6-HYDROXYDOPAMINE INDUCED NORADRENERGIC SPROUTING IN NEONATAL RAT BRAIN. Richard H. Schmidt* and Ranbir K. Bhatnagar (SPON: W.J. Steele). Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

The ability of 6-hydroxydopamine (6-OHDA) to induce regenerative sprouting of noradrenergic (NE) fibers from the locus coeruleus in the cerebellum and brain stem is unique to fetal and perinatal age rats. This study was initiated to define how the capacity of NE neurons to engage in this process changes with increasing age at time of treatment with 6-OHDA.

Female rat pups from several litters were randomized together at birth and assorted into three groups. These groups received 100 mg/kg 6-OHDA subcutaneously on either days 1 and 2 (Group I), 3 and 4 (Group II), or 6 and 7 (Group III). Controls in each group were injected with vehicle. Several rats from Group III were analyzed on day 8, while all the rest were analyzed when 4 weeks old. Pons-medulla and three cerebellar regions were assayed for endogenous NE and synaptosomal NE uptake. The middle cerebellar region consisted of crus I of the hemisphere, vermian lobules VI through VIII, parafloccolus and flocculus. Tissue anterior and posterior to this was taken as separate regions. Areas included in the middle cerebellar region generally mature at a slower rate than the rest of the cerebellum, as judged from granule cell acquisition data (Altman, J. Comp. Neuro. 136:269).

In general, changes in NE and synaptosomal uptake corresponded closely. In those rats analyzed on day 8 degeneration of NE terminals in the whole cerebellum and parietal cortex exceeded 90%. By 4 weeks of age significant sprouting and regeneration was apparent in the brain stem and cerebellum. In Group I NE levels (µg/gm tissue) in the pons-medulla, anterior, middle and posterior cerebellum were 179%, 162%, 148% and 169% of control respectively. For Group II these values were 132%, 80%, 52% and 81%, while for Group III these values were 123%, 21%, 7% and 24%.

The sprouting response in both brain stem and cerebellum is maximal at the earliest treatment time (Group I) and becomes progressively less with maturation. Between day 2 and 4 the NE projection to the cerebellum loses its capacity to regenerate and sprout in excess of control levels of innervation. Nevertheless the capacity for significant regeneration persists in the anterior and posterior regions until at least 6 days of age. This would be particularly evident if the data were to be expressed as total NE per region. At all ages the regenerative potential is greater in the anterior and posterior regions than in the middle. In conclusion, the critical period for regenerative sprouting is clearly different in different target areas of the locus coeruleus, suggesting that target neurons may influence the regenerative process. (Supported by USPHS grant NS-12121)

364 THE DEVELOPMENT OF FIBER PROJECTIONS IN THE OLFACTORY CORTEX OF RATS. J.E. Schwob* and J.L. Price. Dept. Anat. and Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Although the axonal projection of the olfactory bulb onto the olfactory cortex in the rat is not topographically organized, it varies in a systematic way across the cortex; both the thickness of the layer of termination of the axons (layer IA) and the density of termination within this layer are greatest in areas deep to or near the lateral olfactory tract (LOT) and progressively decrease in areas further removed (medially, laterally, and caudally) from the LOT. In an attempt to establish the relationship between this adult pattern and developmental events, the projection of the olfactory bulb has been studied at several foetal and neonatal ages, using the autoradiographic method. At embryonic day 17 (E17), labeled axons can be traced from the main olfactory bulb through the LOT as far as the rostral pole of the medial amygdaloid nucleus. A few are also found in the developing layer I deep to the LOT, but no label is present lateral, medial or caudal to the LOT. At later ages, the labeled fibers gradually extend away from the LOT, although the fringes of the lateral entorhinal area and the medial part of the olfactory tubercle, which receive the lightest projections in the adult, are not innervated by the olfactory bulb until the end of the 1st postnatal week.

Another feature of the adult olfactory cortex is the lack of overlap between the termination of the olfactory bulb fibers in layer IA and that of the cortical association fibers in layer IB. However, experiments with injections into the bulb or the cortex at E21 and E22 indicate that there is considerable overlap between these fiber systems before birth. Injections into bulb or cortex on the 1st postnatal day (P1) demonstrate that the overlap is nearly eliminated in the lateral and posterior parts of the piriform cortex but not in those areas deep to the LOT. The overlap deep to the LOT largely disappears by P5/P6, and a precise, complementary pattern of lamination is established throughout the olfactory cortex by the beginning of the 2nd postnatal week. However, the commissural projection of the anterior piriform cortex to layer IB of the contralateral cortex is not seen until the end of the 2nd postnatal week. Likewise, the projections of the anterior olfactory nucleus to layer IB of the ipsi- and contralateral piriform cortex and the contralateral anterior olfactory nucleus are not seen until the 2nd postnatal week, although the projections to the ipsi- and contralateral olfactory bulb are present at an earlier age.

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365 RADIOAUTOGRAPHIC AND GOLGI STUDIES OF AN EARLY SPINAL CORD REFLEX PATHWAY. Terry J. Sims* and James E. Vaughn. Div. of Neurosciences, City of Hope Med. Ctr., Duarte, Ca. 91010.

Neurons involved in an early reflex pathway of the mouse (C57BL/6J) cervical spinal cord have been studied by radioautography and Golgi impregnations. The objectives of these studies were to establish the final cell birthdays and the timing of subsequent dendritic development for neurons in an early cutaneous reflex pathway. Final cell birthday data were obtained from 6 week old mice which were pulse-labeled at various embryonic times (E8-E15) with ^{3}H-thymidine. Golgi impregnations were obtained for embryonic days 12 through 20.

Lateral motor neurons (LMN) undergo their final cell birthdays between E9 and E10.5, and more than 80% of the total LMN population is generated between E9.5 and E10. The earliest time at which LMN's are stained by the Golgi method is E12. At this time LMN's are identifiable by their ventral root axons, and these young motor neurons characteristically have only two primary dendrites which are directed in the plane of the intermediate/marginal zone interface. Between E12 and E15, LMN dendrites undergo a rapid increase in growth and complexity so that by E16, relatively extensive dendritic patterns have been formed. A few medium-sized neurons located approximately in the medial region of lamina VI are heavily-labeled with ^{3}H-thymidine at E9. Golgi impregnations of medium-sized neurons in this region of E12 spinal cords indicate that these early-formed cells are commissural neurons. Lateral to these commissural neurons in lamina VI are a number of interneurons whose final cell birthdates occur between E10 and E11. Golgi impregnations at E13 show that many of these interneurons send their axons ipsilaterally into the lateral marginal zone and appear to provide an early synaptic input to dendrites of LMN's. Final cell birthdates of dorsal root ganglia (DRG) neurons occur between E10 and E13. A comparison of final cell birthdates with DRG neuronal size indicates that peak generation occurs at E10.5 for the largest neurons, E11.5 for medium-sized neurons, and E12 for the smallest neurons. There is a considerable degree of overlap in the final cell birthdays of neurons in the spinal cord and DRG. However, with the exception of the very early commissural neurons, the neuronal components of the reflex pathway generally undergo final cell birthdays in a sequence that is opposite to the direction of impulse conduction in the reflex arc. The Golgi study supports the radioautographic data in that the earliest impregnations occur in motor neurons and the early commissural neurons, followed by interneurons and finally DRG neurons. Supported by USPHS grant NS 09573.

366 AGING PRODUCES GREATER BEHAVIORAL EFFECTS OF DOPAMINE AGONISTS IN RODENTS. Robert C. Smith, J. Randolph Strong*, D.E. Leelavathi*, and Carolyn Rolsten*. Tex. Res. Inst. Mental Sciences, Houston, Tex 77030.

Biochemical changes in catecholamines and their enzymes have been reported in the aging brain (McGeer et al, Neurobiol. of Aging). Some motor disorders, such as tardive dyskinesia, which may be related to changes in catecholamine neurons and are exacerbated by the DA agonist, amphetamine (Smith et al, Am. J. Psychiat. July, 1977), have much higher prevalence in older age groups. To begin more systematic investigation of the interaction of age and effects of DA agonists, we examined the behavioral effects of apomorphine (APO), a direct DA receptor agonist, and d-amphetamine (AMP) and methylphenidate (MP), indirectly acting DA agonists, in several age groups of rodents: young (3 & 4 month), mature (11 & 14 month) and old (21 & 27 month) rats (Charles River, males) and mice (C57BL/10J, females) respectively. The old rats and mice showed significantly greater gnawing (GNAW) and/or total stereotyped behavior (SB) after APO (0.25-1.0 mg/kg) than young rats or mice, with mature rats or mice generally falling between these two age groups. Old mice also showed greater GNAW after 4.5 mg/kg AMP or 15 mg/kg MP, and old rats tended to have greater SB 90 min after 3.5 mg/kg AMP. Old rats also tended to show slightly greater locomotor activity 30-45 min after 0.5 mg/kg APO. In rats the greatest age difference in the effects of DA agonists tended to occur later in the time course of behavioral effects (e.g. 45 min post APO), whereas in mice the most pronounced age difference in DA agonist effects occurred earlier in the time course (e.g. 15 or 30 min after APO or MP). Since GNAW and SB in rodents are predominantly mediated by nigra-striatal DA, and have been used as models of dyskinetic disorders in man which are hypothesized to involve DA supersensitivity, our results, which indicate greater effects of DA agonists in old rodents, may a) help explain the greater prevalence of dyskinetic disorders in the aged, and b) provide a better animal model for investigating their neuropharmacology. The neurochemical mechanisms underlying the increased effects of DA agonists in old rodents may involve DA receptor supersensitivity and/or decreased metabolism or re-uptake of the agonist drugs or the biogenic amines released by AMP and MP.

367 EFFECTS OF AGE ON EVENT-RELATED POTENTIALS. E. Snyder and S. Hillyard. Dept. Neurosci., UCSD, La Jolla, Ca. 92093.

A number of studies have reported on sensory evoked potential alterations accompanying the normal aging process in man, but most have only recorded from passive, non-performing subjects. Given the wealth of behavioral data which suggest that a number of cognitive deficits are associated with aging and senescence, we decided to look at specific "event related" potentials (ERPs) which are recorded when subjects are actively performing in decision-making tasks. Of particular interest was the "P3" or P300 component, which occurs in normal, young adults when a decision is made regarding a task relevant stimulus.

The subjects studied to date are 5 young (mean age=28yrs.) and 5 older (mean age=71 yrs.) adults. In an initial, non-task condition, the S passively watched a random sequence of slides of the numbers "2" (90%) and "6" (10%) flashed onto a screen. Next, the Ss were instructed to count the "6"s (targets) and to ignore all other stimuli. For this counting task, the stimuli were 80% "2"s (standards), 10% "6"s (targets) and 10% colorful, non-recognizable figures ("novel" slides). In a final condition, Ss were required to press a button to each target "6" (permitting concurrent recording of ERP and reaction time measures).

ERPs were recorded during all conditions from 3 midline, 2 lateral and 2 ocular channels (Fz, Cz, Pz, C3, C4, upper orbit and lower orbit) all referenced to the right mastoid. For each subject, ERPs were averaged separately (using a MED-80 computer) for the standard, target, and novel stimuli in each task condition.

Striking age-related differences were found in certain late components of the ERP. The amplitude of the P3 component to the target "6"s was greatly attenuated in the older subjects. Further, the latency of the P3 peak was delayed by nearly 80 msec in older subjects (despite there being no substantial differences in RTs for the 2 groups). Similarly, the older Ss had significantly smaller P3 components to the "novel" stimuli. Finally, the young subjects showed greater P3 amplitudes at the right lateral electrode site than at the left to the novel slides, while the older subjects had nearly equal amplitudes over left and right hemispheres.

These data tentatively suggest that the ERP component most closely associated with cognitive activity in younger subjects (the P3 wave) is also the most dramatically altered (in amplitude, latency and lateral distribution) by the normal aging process. Further studies will determine the consistency, nature and significance of these age-related ERP fluctuations.

368 LOSS OF AXONS FROM NORMAL AND PERIPHERALLY DEPRIVED TROCHLEAR NERVE. G. S. Sohal and T. A. Weidman*. Department of Anatomy, Medical College of Georgia, Augusta, Georgia 30902.

A loss of approximately half the number of neurons during normal development and a significantly higher cell loss in the peripherally deprived trochlear nucleus of the duck was observed earlier (EN 51:634, 1976). A study of general developmental features and counts of the number of fibers from electronmicrograph montages from day 11 of incubation through hatching and a month after hatching were made to determine whether or not a similar loss of axons occurs during ontogeny of the trochlear nerve. The first appearance of an identifiable trochlear nerve is on day 10 or 11. It is composed of numerous nerve fascicles of small naked axons with an average diameter of 0.4μm. Schwann cells begin to wrap around fibers on day 15 and on day 18 approximately 39% of the fibers are myelinated with an average diameter of 2.0μm. At hatching the myelinated axons (95%) have an average diameter of 3.0μm and the unmyelinated (5%) 0.7μm. A month after hatching 98% of the axons are myelinated.

Initially, there is an abundant collateral sprouting which roughly coincides with the time of neuromuscular contacts suggesting some sort of interaction between the developing nerve and the periphery. The maximum number of fibers (47,000) in the trochlear nerve is present on day 12 and minimum (1500) on day 27 (hatching). Thus, approximately 97% of the fibers are lost during normal development of the trochlear nerve. Axon loss after removal of the superior oblique muscle is even higher. A majority of the axons are lost on days 13-15. It appears that most of the collaterals are retracted into the parent axon and very few myelinated and unmyelinated fibers actually degenerate. Assuming all trochlear neurons send their axons into the nerve, the ratio between cells and fibers on day 12 is 1:20 and on day 13 and after remains 1:1. Cell death slightly preceeds axon loss which suggests that the direction of degeneration is from cell body to the axon. It appears that at least some cells which die during normal development had sent their axons into the nerve prior to their death. Whether these axons make any meaningful connections with the periphery is uncertain. (Supported by GRS Grant 5-S01-RR-05365-14)

369 PRENATAL DEVELOPMENT OF CATECHOLAMINE NEURONS IN BRAIN BY IMMUNOCYTOCHEMICAL LOCALIZATION OF TYROSINE HYDROXYLASE. L.A. Specht*, V.M. Pickel, T.H. Joh, and D.J. Reis (SPON. D.W. Snyder). Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021

Using an immunocytochemical technique for the localization of the catecholamine (CA) synthesizing enzyme tyrosine hydroxylase (TH), we sought to determine in the prenatal rat brain: (a) the earliest time of appearance and the distribution of CA neurons labeled for TH, and (b) the ultrastructure of TH containing axons and subcellular localization of the enzyme. Sections of fixed brain from fetuses of embryonic (E) day 10 to E19 were incubated with specific antiserum to TH and immunocytochemically labeled for light and electron microscopy. By light microscopy, neurons containing TH are first detected on E13. These TH containing perikarya are localized exclusively to the mantle zone of the neural tube. During E13-E17, when labeled axons can be traced from the TH containing perikarya into the marginal zone, labeled processes are also present in the proliferating ependymal zone and are frequently associated with dividing cells in areas of the rudimentary cortex and striatum. At E19, labeled axons and terminals in the proliferating ependymal zone are no longer present. At this time the distribution of labeled cell bodies, processes and terminals is beginning to resemble the distribution of CA neurons in the adult. By electron microscopy, TH labeled axons in the prosencephalon at E17 measure 0.1-0.5μm in cross-sectional diameter, and contain numerous varicosities filled with small (70-100 nm) subcellular organelles which are selectively labeled for TH. The axons showed no synaptic specializations. These results indicate that presumptive CA neurons contain the biosynthetic enzyme TH and may be capable of neurotransmitter synthesis as early as E13. The absence of TH labeled perikarya within the ependymal zone at a time (E13) when CA neurons are undergoing final mitosis (Lauder and Bloom, J.Comp Neurol. 155:469, 1974), suggests that only postmitotic neuroblasts which have migrated to the mantle zone contain TH. The early appearance of perikarya and processes containing TH (E13) and the association of the labeled processes with mitotic cells in the ependymal zone (E13-E17) of the rudimentary cortex and striatum are consistent with a view that the CA neurons may serve a regulatory role in the ontogeny of the central nervous system.
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370 INCORPORATION OF HORSERADISH PEROXIDASE BY DEVELOPING RETINAL CELLS. Arthur W. Spira* (SPON: S.H. Roth). Division of Morphological Science, University of Calgary, Calgary, Alberta, Canada.

Existing studies of the ability of retinal neurons to incorporate extracellular tracers have been limited to the mature retina of lower vertebrates. No comparable data is available for developing and mature neurons of the mammalian retina. Since our previous observations revealed an early development of synapses in the fetal retina of precocious animals we have considered whether vesicles in developing synapses undergo a recycling process at the neuronal terminal similar to that occurring in the mature animal. To this end horseradish peroxidase (HRP-Type II) was introduced to the retina of guinea pigs from fetal to adult ages. (Retinae were dissected from the posterior eye cup; the neural and pigmented epithelium were separated and incubated in 5% HRP in Ames' medium for one hour. Additional animals were injected intravitreously with 30% HRP in saline). During early stages of retinogenesis HRP was incorporated by growth cones and neuron terminals in large vesicles. Differentiating photoreceptor terminals and synapses of the inner plexiform layer contained the tracer in small (700 A) vesicles and tubules. There was an increased uptake by small vesicles by term fetal stages. Animals born and raised in the dark for up to eight weeks continued to show extensive uptake of HRP in photoreceptor terminals. Results are consistent with the postulate that synaptic vesicles in the developing retina recycle in a manner similar to that found in the mature animal.

Tracer was also incorporated by non-synaptic elements. HRP appeared in small vesicles, large vacuoles, and Golgi saccules of ganglion and photoreceptor cells (inner segments). Unlike glial cells of the CNS, Müller cells took up HRP only in their apices. Cells of the pigmented epithelial layer (PE) demonstrated a surprising capacity to incorporate the tracer. In both fetal and mature animals HRP was taken into small vesicles, large vesiculated bodies, and occasionally in branching cisternae resembling those of the smooth ER. This uptake suggests a potential involvement of PE cells in removal of excess subretinal fluid.
(Supported by the Medical Research Council of Canada)

371 ADRENAL EPINEPHRINE AND THE DEVELOPMENT OF TYROSINE HYDROXYLASE ACTIVITY IN SYMPATHETIC GANGLION. Paul Y. Sze and Keith A. Markey* (SPON: S. C. Maxson). Dept. Biobehavioral Sci., University of Conn., Storrs, CT 06268.

In adult mice, bilateral adrenalectomy (Adx) resulted in a 35% reduction of tyrosine hydroxylase (TH) activity in the superior cervical ganglion after 2 weeks. The effect of Adx was shown to be additive to that of decentralization, and was characterized by a decreased V_{max} of the enzyme with no change in the apparent K_m for either tyrosine or 6-MPH_4. The reduction of TH activity following Adx was not prevented by daily replacement with corticosterone (50 umoles/kg, s.c.). However, replacement with epinephrine (22 umoles/kg, s.c., daily) fully maintained TH activity in Adx animals. Daily administration of isoproterenol (22 umoles/kg,s.c.) was equally effective in preventing the decline of TH. Moreover, the effect of Adx could be mimicked by chronic administration of SKF 64139 (420 umoles/kg, s.c.), a drug known to deplete circulating epinephrine by inhibiting adrenal synthesis of the hormone.

The involvement of adrenal epinephrine in the regulation of ganglionic TH was further examined in developing animals. Postnatally, TH activity in the ganglion rises shortly after birth, reaching adult levels at 3 weeks of age. Adx on day 12 was found to arrest the developmental rise of TH activity, while normal development of DOPA decarboxylase was unaltered. Replacement with epinephrine (22 umoles/kg, s.c., daily) and corticosterone (50 umoles/kg, s.c., daily), but not the corticoid alone, permitted the normal developmental rise of TH activity.

These findings indicate that epinephrine not only plays a role in the physiological maintenance of TH activity in adult ganglion, but also serves as an essential factor in the maturation of the enzyme activity in developing ganglion. Thus, in addition to the influence of central innervation, the development of ganglionic TH depends also on hormonal influence of adrenal epinephrine. (Supported by MH 29237).

372 RESERPINE AND/OR α-METHYL-p-TYROSINE INDUCED LESIONS IN BRAINS OF PREGNANT RABBITS AND THEIR FETUSES. Virginia M. Tennyson, Mary Budininkas-Schoenebeck* and John Martin*. Dept. Anat. and Pathology (Neuropathology), Columbia University, College of Physicians and Surgeons, New York, N.Y. 10032.

Pregnant Dutch rabbits were given subcutaneous doses of reserpine (Serpasil, Ciba) and/or α-methyl-p-tyrosine(α-MT, Regis), and their brains and the brains of most of their fetuses were examined by fluorescence and electron microscopy (EM). The adult rabbits weighed 2255-3000 gm. A pregnant rabbit (who aborted her fetuses) received 0.06 mg/day of reserpine from fetal days 14 through 22, and was sacrificed 6 days later. Fluorescence was moderately decreased in the neostriatum. In addition, there was a marked increase in lipofuscin-like granules (autofluorescent bodies in neurons) in her brain, and some areas of focal necrosis containing autofluorescent cells, which were probably lipid-laden macrophages. In another pregnant dam, who was given reserpine (0.038 mg/day) on fetal days 14 through 24, was sacrificed 3 hrs. after the last dose. Fluorescence was absent in the neostriatum of the mother and 8 fetuses. Lipofuscin-like granules were not noticeably enlarged in the mother's brain. EM of the putamen of 2 fetuses showed evidence of damage in neurites. α-MT was given to another dam in multiple doses of 150 mg each three times a day from fetal days 14 through 23, and was sacrificed 1 hr. after the second dose on day 24. This treatment completely depleted fluorescence in the neostriatum of the dam and 6 fetuses, but not in the dam's mesolimbic system. The dam's brain had an increase of lipofuscin-like granules. EM of the putamen of 2 fetuses showed marked damage in some neurites and very large compound granular bodies in neuroblasts. Another pregnant rabbit was given a single dose of 0.125 mg of reserpine in the morning on fetal day 14. That evening and during the next two days, she also received a single dose of 420 mg of α-MT. She died on fetal day 17 and her brain removed soon after death (5 fetuses were present, but were discarded). There was no fluorescence in the neostriatum, mesolimbic system, or substantia nigra. Her brain contained unusually large numbers of greatly enlarged lipofuscin-like granules, particularly in the piriform cortex, dorsal cortex, and neostriatum. This data shows that both reserpine and α-MT given to a pregnant rabbit during a critical period during the development of dopaminergic neuroblasts can produce alterations in the fetal putamen, and in higher doses result in lipofuscin-like granules in the brain of the dam.

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373 THE EFFECT OF NERVE GROWTH FACTOR ON REGENERATIVE REPAIR IN THE SEVERED OPTIC NERVE OF THE NEWT (TRITURUS VIRIDESCENS). James E. Turner and Kathleen A. Glaze*. Dept. Anat., Bowman Gray Sch. Med., Wake Forest University, Winston-Salem, N. C. 27103.

Nerve growth factor (NGF) was found to have a strong stimulatory effect on the regenerative capacity of the lesioned newt (Triturus viridescens) optic nerve. A clear dose response relationship was demonstrated to exist between various concentrations of single intraocular NGF injections, administered at the time of lesion, and the number of regenerating axons per nerve cross section at 14 days post lesion. A rapid rise in numbers of regenerating axons was evident in animals treated with NGF concentrations of 2 to 20 B.U. but values plateaued between 20 - 2000 B.U. Light microscopic observations indicated that NGF treated nerves were larger than controls. Quantitative analysis substantiated these initial observations by revealing that a single intraocular injection of 200 B.U. of NGF given at the time of lesion elicited a highly significant increase in both diameters and cross-sectional areas of regenerating nerves 14 days after lesion. Most importantly, NGF treatment elicited approximately a two-fold increase in the total number of regenerating axons per nerve cross-section. In addition, the percentage of nerve cross-sectional area occupied by regenerating axons was significantly increased by NGF treatment. However, the percentage of glial cytoplasm per cross-sectional area remained constant for both control and experimental groups.

374 NERVE-FREE DEVELOPMENT OF MERKEL CELLS IN AMBYSTOMA EMBRYOS. Charles D. Tweedle. Depts. Biomech. and Zool., MSU, East Lansing MI 48824.

At stage 24 (before neural crest migration) aneural Ambystoma maculatum embryos were prepared by surgical removal of all presumptive neural tissue. These experimental animals as well as normal embryos were then allowed to develop in sterile Steinberg's solution. At intervals throughout development sample animals were fixed for electron microscopy and thin sections taken from the digits of the forelimb. Recognizable Merkel cells (mechanoreceptors) were found in all notch stage limbs. At this time, many of the Merkel cells had not acquired a fully differentiated appearance and in normal embryos only 5-10% of the Merkel cells had become innervated. By the four digit stage all of the Merkel cells in normal embryos were innervated. Even after 2-3 months of nervelessness, the aneural Merkel cells showed no obvious ultrastructural signs of breakdown; cell size and the number of dense core granules per cell were not statistically different from normal. In this case, it would thus seem that sensory receptor development and maintenance are not nerve dependent.

375 CHOLINERGIC INHIBITORY MECHANISM MATURES EARLY IN RAT. Carol Van Hartesveldt, F.-C.T. Chang*, and William Guido*. Dept. Psychol., U. Fla., Gainesville, Fla. 32611

The maturation date of a cholinergic system inhibiting behavior has been set at 20 days postnatal, based on evidence that scopolamine injected peripherally increases activity beginning at that age (Campbell et al., 1969). However, in the present experiment, scopolamine injected intracerebrally increased locomotor activity at 2 days postnatal. We injected 10 µg scopolamine in .2 µl saline, 20 µg scopolamine in .2 µl saline, or .2 µl pH-adjusted saline unilaterally or bilaterally into the caudate nucleus of 2-day old rats. We directly observed locomotor behavior for 30 minutes immediately after injection. Rats given scopolamine made significantly more coordinated "walking" movements of all four limbs and tail than did rats receiving saline. We conclude that a forebrain inhibitory mechanism is mature as early as 2 days postnatal in the rat, but cannot be demonstrated behaviorally by peripheral administration of scopolamine until later.

376 DEVELOPMENTAL CHANGES IN SYNAPTOSOMAL UPTAKE OF GAMMA AMINOBUTYRIC ACID IN RAT CEREBRAL CORTEX. A.D. Vanker M.B. Friend* and G.E. Duncan*. Biol. Dept., Georgia State Univ., Atlanta, GA, 30303.

The Na^+-dependent, high-affinity uptake of ^{14}C-gamma-aminobutyric acid (GABA) by suspensions of crude synaptosomal fractions derived from adult female and neonatal (1-3 days old) were measured.The incubation media were modified tris buffers containing either a high concentration (108mM, HNa) or low concentration (18 mM, LNa) of sodium ions. Each experiment was performed at six temperatures ranging from 10^o to 40^o. Cleland's computer program for enzyme kinetics analysis was used to determine the apparent V_{max} and K_m for GABA uptake. In all cases, V_{max} was directly proportional to temperature and was higher in the adult than in the neonate for both HNa and LNa. K_m values for the adult in HNa varied from 3.43 µM at 10^o to 6.91 µM at 40^o; in LNa the range was 5.71 to 8.47 µM. For the neonate, K_m varied from 1.50 to 3.45 µM in HNa and from 3.01 to 3.27 µM in LNa. However the increase in K_m was directly proportional to temperature only for the adult in HNa. Arrhenius plots using the values of V_{max} indicated that the activation energies (E_a) were similar for the adult and the neonate in both media although higher in HNa. van't Hoff plots of K_m indicated that, while the ΔH_m (the energy acquired during the initial interaction of GABA with the transport mechanism) was essentially the same for the adult (4.30 kcal) and the neonate (4.33 kcal) in HNa, ΔH_m were quite different in LNa (adult: 2.60 kcal; neonate: 0.7 kcal). These and other analyses indicate that the Na^+-dependent, high-affinity transport (uptake) of GABA by presynaptic neuronal elements undergoes changes during maturation, and that this transport process is more sensitive to sodium ion concentration in neonatal preparations.

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377 THE PENTOSE PHOSPHATE PATHWAY IN REGENERATING SKELETAL MUSCLE. K.R. Wagner*, S.R. Max, F.C. Kauffman and C.L. Koski*. Depts. Neurology, Pediatrics, and Pharmacology and Experimental Therapeutics, Univ. Maryland Sch. Med., Baltimore, MD 21201.

Glucose-6-phosphate dehydrogenase (G6PDH) appears to be rapidly induced in rat skeletal muscle following injection of Marcaine, a myotoxic local anesthetic which causes widespread degeneration followed by regeneration of muscle fibers (Fed. Proc. 36:922, 1977). We have extended these studies on G6PDH and have measured the activities of additional enzymes of the pentose phosphate pathway, viz., the oxidative enzyme, 6-phosphogluconate dehydrogenase (6PGDH), and the non-oxidative enzymes, transketolase, transaldolase, ribose-5-phosphate isomerase and ribulose-5-phosphate-3-epimerase. The specific activities of G6PDH and 6PGDH increased 2 h after a single intramuscular injection of Marcaine, reached 6 times control by 8 h, and 9 times control by 24 h. Actinomycin D and cycloheximide both prevented the increase. Enzyme activities of mixed supernatant fractions from experimental and control muscles were additive. In addition, kinetic analysis revealed a marked increase in Vmax of G6PDH and 6PGDH, but no change in apparent Km. Isoelectric focusing of G6PDH demonstrated a single band with the same isoelectric point in supernatants from experimental and control muscles. The intensity (per mg protein) of the band increased progressively with time following Marcaine injection. These results support the hypothesis that enhanced activities of G6PDH and 6PGDH are attributable to synthesis of enzyme protein. Changes in activity 8 h after Marcaine treatment partly reflect infiltration of muscle by phagocytic cells as indicated by light microscopy and increased β-glucuronidase activity. The specific activities of the non-oxidative enzymes also increased following Marcaine injection, but to a much smaller extent than the oxidative enzymes. We postulate that increased activity of the pentose phosphate pathway is important for anabolic processes in the initial stages of muscle regeneration. (Supported by NIH grant NS 31142 and by the Muscular Dystrophy Assn., Inc.)

378 NEUROCHEMICAL CHANGES FOLLOWING PRENATAL IRRADIATION OF SQUIRREL MONKEYS. Lary C. Walker* and B. Kaack (SPON: A.A. Gerall) Delta Primate Center, Covington, Louisiana 70433.

According to tentative but widely used standards, any human fetus exposed to 10 rads or more during early pregnancy should be therapeutically aborted to avoid the possibility of a physically and mentally retarded child. Thus far, most experimental work on prenatal radiation effects on the brain has been done with rodents. For comparative reasons, increasing interest has been directed toward the squirrel monkey as a diurnal primate model for studying the effects of such environmental hazards as radiation on the development of brain mechanisms and behavior. The objectives of this study were to examine the effects of 10 and 100 R whole-body Co-60 irradiation during pregnancy on neurological maturation, sensory-learning-motor performance in relation to neurochemical and morphological changes in specific cortical and subcortical regions of the offspring brain at 30, 365 and 730 days of postnatal life. More specific aims of this phase of the study were to examine the effects of prenatal radiation on neurotransmitter and hormone interactions regulating specific categories of physiology and behavior.

Infant squirrel monkeys sacrificed at 30 days postnatally showed a slight dosage dependent decline in total protein levels across nine separate brain areas. In addition, the levels of the radiosensitive enzyme glycerolphosphate dehydrogenase were generally higher in the cortices of irradiated animals than in the controls. The magnitude and direction of prenatal irradiation effects on the cholinergic synthesizing enzyme, choline acetyltransferase and the hydrolyzing enzyme, acetylcholinesterase, depended upon the brain area studied and the time of prenatal irradiation. $Na^+ - K^+$ ATPase activity in three brain regions was compared among the control, 10 R and 100 R groups, and similar comparisons were made of the circulating hormones cortisol, T_3 and T_4. These changes in relation to behavior, physiology and morphology are currently contributing to the development of a clinical profile of the primate infant exposed in utero to Cobalt-60 radiation. (Supported in part by NIH Grant 1-R01-HD09942).

379 β-ADRENERGIC RECEPTOR: PRESENCE IN CORTEX AND CEREBELLUM OF NEWBORN RAT. Kenneth G. Walton, Edie Miller*and Ross J. Baldessarini. Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Much evidence now supports the idea that the interaction between a β-adrenergic receptor and adenylate cyclase constitutes a portion of the postsynaptic response mechanism in noradrenergic neurotransmission. This idea is upheld by studies in brain as well as in the periphery. However, one area where the evidence in brain is not yet clear is the time of onset of β-adrenergic receptor-cyclase activity in the young rat. The iontophoretic studies of Woodward et al. (Brain Res. 34:73, 1971) appear to indicate that cerebellar Purkinje cells of the one-day-old rat are capable of responding to norepinephrine (NE) by the same cyclic AMP-mediated mechanism as the Purkinje cells of older rats, even though it is apparent that Purkinje cells of the newborn have not yet received their noradrenergic input from the locus coeruleus. Both this work and the recent studies of Coyle and Molliver (Science 196:444, 1977) showing what seem to be functional noradrenergic synapses in the cerebral cortex of newborn rat suggest that the cerebellum and cortex of the newborn should contain β-adrenergic receptor-cyclase to be consistent with the hypothesized role of this enzyme system in the postsynaptic response to NE. In earlier attempts to answer this question Schmidt et al. (Dev. Psychobiol. 3:53, 1970) found stimulation of cyclic AMP levels by NE in slices of whole brain only after 6 postnatal days, while Perkins and Moore (Mol. Pharmacol. 9:774, 1973) and Harden et al. (Brain Res. 125:99, 1977) found little evidence for stimulation of cyclic AMP synthesis in slices of cerebral cortex before the 5th postnatal day. Using minced preparations in the presence of the potent phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), and measuring cyclic AMP directly by a binding-protein assay, we have found as much as 10-fold stimulation of cyclic AMP levels in cortex and cerebellum from one-day-old rat in response to 1 μM of the β-adrenergic agonist isoproterenol. Stimulation in the absence of IBMX was not always observed, but was sometimes as high as 3-fold. Reasons for the apparent descrepancy with the earlier observations are not clear, but probably are related to the different conditions of incubation and methods of assaying for cyclic AMP. The presence of the β-adrenergic receptor-cyclase system in cerebral cortex of newborn rat appears to support other evidence that noradrenergic synapses are functional at this time and thus is consistent with the possibility that noradrenergic pathways are involved in subsequent stages of cortical development, as suggested by the studies of Kasamatsu and Pettigrew (Science 194:206, 1976). The presence of this enzyme system in cerebellum at one day is consistent with the above-mentioned evidence that the receptor mechanism preceeds noradrenergic synapse formation on cerebellar Purkinje cells, a finding of potential importance to understanding the factors controlling synapse formation generally.

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380 UNCROSSED RETINOGENICULATE PROJECTIONS OF ALBINO VS. PIGMENTED A/J-+/c(N7) MICE. Irwin S. Westenberg. V.A. Hosp., Phoenix, AZ 85012.

A developmental abnormality associated with albinism in mammals involves reduced uncrossed retinogeniculate projections (RGP). The effects of mutant genes at the "C", or albino, locus are clearest when coisogenic albino and pigmented animals are compared. To date this approach has been applied only in the case of pigmented inbred strains of mice in which a spontaneous mutation to albinism has occurred. However, any inbred strain may be used; first an inbred albino is crossed with a pigmented animal, or a pigmented inbred animal is crossed with an albino. Then offspring of the cross are backcrossed to the inbred strain. Offspring of the backcross that carry one "+" (wild-type) gene and one mutant "c" (albino) gene at the C locus are selected for the next backcross to the inbred strain. Each subsequent backcross increases the coefficient of inbreeding. This technique is being applied by Wahlsten; he is introducing a "+" gene in the place of a mutant "c" gene at the C locus of inbred albino mice of the A/J strain. An initial cross, A/J x DBA, resulted in pigmented offspring. Each backcross to A/J resulted in albino and pigmented pups; the pigmented pups were always chosen for the next backcross. I have examined the uncrossed RGP of two littermate pairs from Wahlsten's seventh backcross to A/J, designated A/J-+/c(N7); their coefficient of inbreeding is 0.992. Each pair consisted of an albino and a pigmented littermate of the same sex; one pair was male (341 days old), and the other was female (350 days old). The right eye of each mouse was removed, and six days later the mice were perfused. Horizontal frozen brain sections were stained for degenerating optic tract processes and examined on slides coded to conceal each mouse's genotype. In each pair the albino's uncrossed RGP were smaller than those of its pigmented counterpart. The effects were similar to those observed in other, unrelated strains. Given the small number of subjects and the less-than-optimal coefficient of inbreeding, these results are preliminary. The important point is that anomalies associated with albinism can be studied with appropriate genetic controls in many species; one need not wait for spontaneous mutations in inbred strains. Limiting factors include the time required to produce a sufficient number of backcross generations (preferably 10 or more), the risk of reduced vigor with successive backcrosses, and the species for which inbred strains are currently available (rabbit, mouse, rat, guinea pig, hamster, dog, cat, etc.).

Mice were bred, enucleated and perfused in the laboratory of Dr. D. Wahlsten. Histology was performed by C. Kalaha-Brunst.

381 ACTION POTENTIAL MECHANISMS IN PROCESSES OF AMPHIBIAN NEURONS DEVELOPING IN CULTURE. Alan L. Willard* (SPON: S.L. Foote). Biology Dept. UCSD La Jolla, CA. 92093.

In mature neuronal somata the inward current of action potentials (APs) is carried by Na^+, Ca^{++}, or by both ions. In contrast, most neurites have propagated APs in which Na^+ carries the inward current. During development the ionic basis of somatic APs in amphibian neurons changes. The inward current is carried by Ca^{++} at stages when the cells first become excitable, by both Na^+ and Ca^{++} at later stages, and finally by Na^+ alone (1). The same sequence of changes in somatic excitability has been seen in vitro (2). The development of excitability in neurites has not been previously examined. Here I report that APs in neurites of cultured amphibian neurons depend on Na^+ at a time when somatic APs depend on Ca^{++}.

Cells from neural plates of Xenopus laevis were dissociated by exposure to Ca-Mg^{++} free saline and grown in sterile Steinberg salt solution plus 0.1% Bovine serum albumin as previously described(2). Isolated neurons could be recognized by 6 hrs after plating. A single microelectrode was used to record intracellularly from the soma. APs were elicited both by current injection through the recording electrode and by extracellular stimulation of neurites with a glass pipet (2μm tip). Culture dishes were continuously perfused with salines of varying ionic composition.

Intracellular current injection produces 2 kinds of regenerative response in 12-20 hr old cultures. A larger one which appears to be the somatic AP overshoots zero, is as long as 600 ms in duration, and is blocked by 1 mM La^{3+}. Stimuli just at threshold also produce a smaller, briefer response without the larger one. Extracellular stimulation of the neurites leads to regenerative responses of the same amplitude and duration as this smaller response. Unlike the somatic AP, this response is blocked by Na^+ free saline. The latency of this response depends on the length of neurite between the soma and the stimulating pipet and it disappears when the pipet is displaced from the neurite by as little as 10 μm. In some cases neuritic stimulation produces an AP which appears identical to the large somatic AP, presumably due to excitation of the soma by the neuritic AP.

These results indicate that the inward current of APs propagated along neurites of cultured cells depends chiefly on Na^+ at a time when the somatic AP depends on Ca^{++}. Thus the Ca^{++} phase of membrane excitability either occurs at a different time or it does not occur at all. It seems clear that somatic and neuritic membranes are regulated separately during development. It will be of interest to study the control mechanisms by which this regulation is achieved. (Supported by NSFBNS 76-08348 & NIHUSPHS 07153-01). 1. Spitzer& Baccaglini (1976) Br. Res. 107 610-616.
2. Spitzer & Lamborghini (1976) P.N.A.S. 73 1641-1645.

382 AGING THEORIES: TESTS BY TWO-DIMENSIONAL PROTEIN MAPPING OF NERVOUS TISSUE. David L. Wilson, Michael E. Hall, and George C. Stone. Dept. of Physiol. and Biophys., Univ. of Miami, Sch. of Med., Miami, Fl. 33152.

An organism and its neurons are about the same age. Therefore, nervous tissue is especially useful for tests of aging theories. Using O'Farrell's (1) two-dimensional gel electrophoresis technique for protein analysis, with some modifications, we have compared the protein patterns for superior cervical sympathetic ganglia (SCSG) for young and old rats. Male Fisher rats with mean longevity of 640 days (2) were used.

Such aging hypotheses as error theories, the cross-linkage theory, the free-radical theory, the mutation theory, and the autoimmune theory predict changes in proteins or protein synthesis with aging. In brief, error and mutation theories predict charge changes (from amino-acid substitutions) in proteins; cross-linkage and free-radical theories predict increases in molecular weight (due to covalent linkages) of proteins; and the autoimmune theory predicts reduction or absence of particular protein species.

SCSG from young (2-4 mo.), adult (9-11 mo.) and old (22-24 mo.) rats were incubated in vitro for one hour in the presence of ^{14}C-leucine prior to protein extraction for two-dimensional mapping. Proteins were separated according to isoelectric point in one dimension and molecular weight in the second dimension. The changes predicted by the above aging theories were detected neither in the staining pattern, showing protein amounts, nor in the labeling patterns on autoradiographs of dried gels, showing newly-synthesized proteins. It is possible that other tissues would show such changes or that the kinds of changes predicted by the theories were too minor to be detected. Nevertheless, the results suggest that, at least for rat, some other mechanisms for aging are present.

Supported by NIH grant NS12393 and by a Biomedical Research Support Grant. MEH is a postdoctoral trainee (NS 7044). We thank M. Rockstein for the Fisher-strain rats.

(1) O'Farrell (1975) J. Biol. Chem. 250, 4007.
(2) Chesky and Rockstein (1976) Exper. Aging Res. 2, 399.

383 AGING AND ADAPTATION OF DRINKING RHYTHMS IN RATS.
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In a first study (Wolthuis et al., Neurosci. Letters 2, 243, 1976) acquisition of positively or negatively reinforced suppression of drinking was found to be deficient in old (30 months) rats compared with adult (18 and 12 months) or young (3 months) rats. The test situation was low in interference level, required little motor skill and measurements indicated that the deficits found could not be attributed to age-differences in activity.

The question arose whether age-related disturbances could also be demonstrated in more basic CNS-functions, such as in adaptation of diurnal or circadian rhythms of drinking activity to changes in lighting conditions.

Diurnal and circadian variations in drinking behaviour were investigated in two experiments with female rats of 3, 12 or 30 months old. After 6 days of a standard 12/12 h lighting schedule in both experiments, the rats were either exposed to a reversed light-dark lighting schedule or to continuous lighting for 18 days. Thereafter, they were again exposed to the standard 12/12 h lighting schedule for another 10 days in both experiments. The number of drink responses was sampled each 3 hours throughout the duration of the experiments.

Rats adapted their drinking behaviour rapidly when light-dark conditions were reversed and subsequently changed back to normal. No differences were found between the three age groups. When the normal light-dark conditions were replaced by continuous illumination, drinking activity showed a free-running circadian rhythm. In the young (3 months) and the adult (12 months) rats the period length of this circadian rhythm was 25.0 - 25.5 hours, whereas in old (30 months) rats the period length remained closer to the original 24 hours. In all animals the free-running rhythms persisted for at least 18 days, although the amplitude decreased. When lighting was switched back to the standard 12/12 h schedule, the young and the adult rats returned more quickly to their normal rhythms with a normal amplitude than the old rats.

It is concluded that, when the "Zeitgeber" is absent, old rats keep more closely to a pre-existing rhythm than young and adult rats, which suggests a greater behavioural rigidity in aged rats. In addition, old rats exhibit a greater negligence in drinking behaviour and a changed rest or sleep-wake pattern.

384 EFFECT OF CHRONIC INGESTION OF TRITIATED WATER ON PRENATAL BRAIN DEVELOPMENT.
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In view of the anticipated increased use of atomic energy in industry, we studied the possible long term effects of chronic radiation exposure. Female rats were given tritiated drinking water [^{3}HOH; 3 μCi/ml (lower dose) or 30 μCi/ml (higher dose)] from adolescence (60 days) and throughout pregnancy resulting in a total exposure to 3.6 or 32.4 mCi over 54 days. No signs of radiation illness were observed. Gestation was prolonged at both dose levels. The ovulatory process (litter size) and pregnancy maintenance rates were normal. However, at both doses, the newborns exhibited highly significant decreases in body and brain weight, and brain cell number and protein, as well as increased vascular fragility (hemorrhages).

^{3}HOH administration at the respective doses was then continued throughout weaning and adolescence. Only during the first ten days of life (in F_1) a high mortality (33%) was found at the higher dose. At 30 days the decreases in protein content of cortex, midbrain and cerebellum were up to 37%, and in the cortical cell population up to 13%, indicating a severe reduction in cell size as well. At 90 days some rehabilitation in cortical weight and its total cell number was observed; yet, the midbrain and cerebellum remained decreased in these two parameters, for both dose levels. Protein synthesis remained severely inhibited in all brain regions.

Administration of ^{3}HOH was then continued at both dose levels throughout pregnancy. At the lower dose, all females had normal pregnancies. At the higher dose, a high incident of failure to maintain pregnancy, a significant decrease in litter size, and high (23%) incidence of still births was encountered. At birth, brain development of surviving offspring (F_2) was again highly significantly decreased at both dose levels. However, there was no more damage in F_2 than in F_1, i.e. there was no cumulative effect of radiation; high mortality and a selection for individuals less susceptible to radiation may be implicated.

All male offspring (F_1 adults) exposed to the higher dose were sterile, but there was no decrease in male fertility at the lower dose. The rate of accumulation of ^{3}HOH in maternal blood and rapidly multiplying tissues (placenta, fetuses) and in the amniotic fluid has been determined. Ova maturation and cell divisions in animals following chronic administration of ^{3}HOH have also been investigated. (Supported by ERDA grant E(04-3)-34.)

385 ACTIVITY IN THE DEVELOPING LATERAL LINE OF THE TADPOLE OF XENOPUS LAEVIS.
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Neuroscience Dept., UCSD, La Jolla, CA 92093.

The peripheral portion of the lateral line system in Xenopus consists basically of: 1)hair cells which transduce vibrations in water, 2)axons, whose cell bodies are centrally located, which transmit either afferent or efferent signals, and 3)synapses which carry the signals between hair cells and axons. Two questions about the development of the system are: When is it first active? What are the changes in its physiology over time? The preparation consists of an intact Xenopus tadpole whose post-orbital lateral line nerve has been exposed between the brain and the first sensory organ by means of a small window in the dorsal surface of the animal. The tadpole is immersed in Ringer's solution and viewed through a compound microscope. A suction electrode is used to record activity in the nerve. Stimulation is provided by a gated sinusoidally-vibrating needle applied to the sensory hairs.

The earliest stage at which activity has been previously reported is Nieuwkoop & Faber stage 54 (NF54)(26 days)(Shelton, JEEM 1970). Nieuwkoop & Faber state that the "lateral line system [is] becoming externally visible" at NF43 (Normal Table, 1967). I observe sensory hairs as early as NF41 and record afferent responses at the same time. I see efferent activity, evoked by stimulating another lateral line, as early as NF44. I also record spontaneous activity at these early stages.

Changes are seen in several properties. The duration of the action potential, as measured by the time between zero-crossings of the largest component of the tri-phasic potential recorded from afferent fibers of good signal-to-noise ratio, decreases from 2.3 ± .3msec at NF41 to 1.1 ± .1msec at NF47. The frequency response, measured as the highest frequency at which phase locking is seen, increases from approx. 100Hz at NF41 to >300Hz at NF47. Spontaneous activity increases as a function of stage; at early stages it is rarely seen and of low frequency if present.

The lateral line is thus responsive to stimulation at an early stage, apparently as early as the appearence of the sensory hairs. Since Chung et al. (Proc. R. Soc., 1974) were able to record visually evoked responses from the tectum no earlier than NF43, the lateral line appears to be the first system used to sense the distant environment. Several properties of the system change during development; in particular, the increase seen in frequency response indicates an increased sensitivity. Further investigation may reveal how each of the three elements are involved in producing these changes.
(Supported by NSF BNS 76-08348 and NIH USPHS 07153-01).

EEG AND EVOKED POTENTIALS

386 EFFECTS OF DISCRETE ELECTROLYTIC LESIONS ON SCALP-RECORDED AUDITORY BRAINSTEM RESPONSES. Joseph Achor* and Arnold Starr. Depts. of Psychobiology and Medicine, UCI, Irvine, CA 92717.

Discrete electrolytic lesions were made in acute experiments on adult cats to provide information about the generation of scalp-recorded auditory brainstem responses. Repeated recordings of monaural "click" evoked responses were obtained from a vertex-neck configuration for one hour prior to and following each lesion to ensure stability of lesion effects. Most of the lesions produced multiple effects in terms of amplitude changes. Few latency changes occurred. Determination of the generators was complicated by the lack of specific knowledge about which auditory structures were affected by the lesions. Most of the lesions interrupted fiber tracts as well destroyed nuclear structures. The most significant findings were the absence of evoked response changes with lesions of certain structures. A lesion in the cochlear nucleus which was confined to the dorsal division had no effect on the Auditory Brainstem Response. In another animal a lesion occupying about 50% of the central nucleus of the inferior colliculus produced no waveform changes during the 9.8 millisecond analysis epoch. A second lesion in that animal which destroyed two-thirds of the opposite central nucleus of the inferior colliculus produced minimal effects. No effect was seen with contralateral stimulation and only a small amplitude decrease in the peak at six milliseconds (Component 5 of Buchwald and Huang, 1975) was observed to ipsilateral stimulation. These results suggest that structures caudal to the inferior colliculus are the generators for the Auditory Brainstem Response. The absence of any effect with a lesion of the dorsal cochlear nucleus suggests that some of the elements of the auditory pathway do not contribute significantly to the scalp recording, even though they are positioned in the beginnings of the pathways.

387 ELECTROPHYSIOLOGICAL CONCOMITANTS OF THE "COROLLARY DISCHARGE" IN THE VISUAL SYSTEM.+ John S. Barlow and Janet Dubinsky. Neurophysiol. Lab, Neurol. Serv., Mass. Gen. Hosp., Boston, MA 02114.

In a multi-faceted study of oculomotor and EEG aspects of the "corollary discharge" in the visual system, postulated to maintain stability of the perceived visual world despite saccadic eye movements, recordings have been carried out under a number of different experimental conditions on a series of human subjects. Eye movement (monitored electro-oculographically and/or by the infrared technique /Biometrics eye-movement monitor/, multi-channel EEGs, and stimuli were recorded on magnetic tape (Ampex PR2200, 1/2-inch tape, 16-channel) or on an improved 24-channel cassette system (Electroenceph. clin. Neurophysiol., 1975, 38:183-186).

Preliminary findings include the following: (1) concordance between perceived direction of afterimage and actual eye position, when eyes open in the dark, as contrasted with discordance when eyes are closed, when gaze is alternated; the perceived excursion of the afterimage remains much the same whereas the excursion of the eyes tends to be larger and less precise when eyes are closed (a difference not ascribable to the EOG technique), suggesting a partial "decoupling" of oculomotor control with eyes closed in contrast to the effect of the "corollary discharge" on the direction of the perceived afterimage; (2) a more limited occipital distribution, and briefer duration, of potentials associated with horizontal alternation of gaze at a stationary pattern of vertical stripes or squares than when the pattern is shifted and gaze remains fixed; (3) results for congruent pattern shift with alternation of gaze (i.e., position of pattern controlled by eye position) resembles those for alternation of gaze alone rather than those for pattern shift alone; (4) a different form and distribution of potentials during reading (e.g., an occipital P300 wave) from that during alternation of gaze; (5) no EEG potentials associated with saccadic eye movements occur during alternation of gaze in total darkness, nor during simulated reading, whether or not an afterimage is present; (6) brief short-latency potentials limited to the occiput appear during the small rapid oscillatory eye movements of voluntary nystagmus, while viewing a patterned visual stimulus.

These findings tentatively suggest, among other conclusions, that (1) the "corollary discharge" in the visual system may already be operative in those parts of the visual cortex sampled by electrodes at the occiput; (2) its electrical manifestation is contingent upon an actual visual input, and (3) its end effect at a perceptual level is independent of whether the eyes are closed or open. (+USPHS Research Grant No. NS 03752)

388 THE RELATIONSHIP OF EVOKED POTENTIAL CHARACTERISTICS TO TIME PERCEPTION JUDGEMENTS.[1] Ernest S. Barratt, Perrie M. Adams and James H. White.* Dept. of Psychiatry, University of Texas Medical Branch, Galveston, Texas 77550

Twelve adolescent males were studied in a psychophysiological procedure which involved time perception judgements and visual evoked potentials to different levels of stimulus intensity. The time perception judgements involved 3 modes: Estimation (subject estimates the length of time, 1 to 10 seconds inclusive, a light was presented to him), Reproduction (subject reproduces the interval of time just shown to him by the experimenter) and Production (subject produces the interval of time requested by the experimenter without giving a model of the interval). Each of the 10 time intervals was presented 3 times under each mode. All the trials for a given mode are presented together in a randomized sequence. Between the time judgement modes, visual evoked potentials were recorded at the vertex under different intensity levels. The subjects were then classified as either "augmenters" or "reducers" according to changes in the amplitude of the P100-N140 component across the 3 intensity levels. Comparison of time judgements for the 3 modes was then made on the basis of these evoked potential groups. Reducers (N=6) were found to be more accurate regardless of the judgement mode than were the augmenters (N=6). In addition, reducers would overproduce a given interval and underestimate the interval while the opposite was true for the augmenter. These results suggest that time perception judgement is sensitive to neurophysiological differences observed between individuals and should prove useful in the study of the relationship of cortical potentials to processes involved in attention and memory.

[1]Supported by grant from Office of Naval Research, Physiology Division.

389 SOMATICALLY EVOKED MAGNETIC FIELDS FROM THE HUMAN BRAIN. D. Brenner*, J. Lipton*, L. Kaufman*, and S.J. Williamson*.(SPON: D. Yager). Neuromagnetism Lab., Dept of Physics and Psychology, NYU, N.Y., N.Y. 10003.

A magnetic field produced by electrical currents flowing in the human brain in response to a somatic stimulus has been detected for the first time. Repetitive transcutaneous electrical stimulation of a finger evokes a magnetic field on the contralateral side of the head. This field has been detected with a superconducting detector in an unshielded environment. The field pattern was mapped for stimulation of the little finger and to a first approximation is qualitatively similar to what would be produced by a current dipole source. The dipole would be located near the post central gyrus, oriented parallel to the scalp and perpendicular to the Rolandic fissure. Its location agrees with the location of the projection of the little finger on the somatosensory cortex (the "sensory homunculus"). Stimulation of the thumb produces a 2cm downward shift in the field pattern, again in agreement with the sensory homunculus.

Responses at the fundamental frequency for stimulus repetition rates between 18 and 30 Hz show little intersubject variability and can be characterized by a response latency of 70 ms. Between 3 and 18 Hz, subject responses show large differences. Motor reaction times of a simple pulse had an average value of 172ms. Based on a serial model, this gives a motor response time of 102 ms, a value close to the motor response time of 115 ms calculated for the visual system. (1) The significance of the variation of responses as a function of frequency is discussed in terms of fast and slow neuron types.

(1) Williamson, S.J., Kaufman, L., Brenner, D. Vis. Res., in press.

390 LONG-TERM CHANGES IN P3 AMPLITUDE AND SCALP DISTRIBUTIONS WITH EVENT REPETITION. Rachel Y. Courchesne*, Eric Courchesne, and Leo Ganz (SPON: J. M. Ford). Dept. of Psych., Stanford U., Stanford, CA 94305.

There are currently several reports on short-term changes in P3 amplitude with event repetition. Courchesne et al (1975) found a decrease in amplitude of frontal P3 waves elicited by repeated nontarget stimuli; Woods et al (1976) found an increase in amplitude of target P3 upon frequent event repetition, while Squires et al (1976) found a decrease in amplitude of P3 waves when target events are repeated with little or no interpositions. There are, however, no reports on long-term changes of P3 amplitudes with event repetitions. This paper studies long-term changes in the amplitude of P3 waves to target and nontarget events with event repetitions.

Subjects counted the number of presentations of target letter Bs (p = .10) which were interposed randomly in a sequence of tachistoscopically flashed background slides (letter As; p = .80). In some sequences, nontarget novels (unrecognizable slides; p = .10) were presented, while in others, nontarget dims (easily recognized, small letters C to Z; p =.10) were presented. The targets elicited high amplitude P3 waves maximal at Pz; neither P3 amplitude nor scalp distribution changed significantly across the first 16 target presentations. However, P3 waves to the dims and novels changed significantly. Dim events always elicited P3 waves, maximal at Cz and Pz. No significant changes in scalp distribution were found. However, P3 amplitude at all electrode sites (Fz, Cz and Pz) decreased at similar rates across the first 16 dim presentations. The P3 waves to the novel events, on the other hand, changed in complex ways. On the initial 3 trials, P3 waves were maximal over the frontal cortex. With each successive novel presentation, the P3 amplitudes decreased frontally and increased parietally; until, after 5 to 8 presentations, they were maximal over the parietal cortex. The data support the notion that there are several distinct types of P3 waves. The differences in the scalp distributions of P3 waves to novel and dim events apparently correspond to different modes of evaluating event content -- a "frontal" mode to process initially unrecognizable events and a "parietal" mode to process initially easily recognized events. With successive presentations of novel events, subjects may be developing "models" of these novel events, and, correspondingly, they may be employing successively different modes to process them. The changing scalp locus of maximum P3 activity may reflect these dynamic changes in the processing of the novel events.

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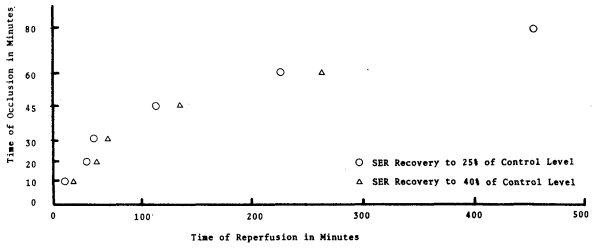
391 RECOVERY OF CORTICAL EVOKED RESPONSES AFTER CEREBRAL ISCHEMIA. Robert J. Gaudet* and K.M.A. Welch (SPON: P. Kellaway). Dept. Neurol., Baylor Coll. Med., Houston, Tx. 77030.

The ability of cortical electrophysiological activity to survive and recover from ischemia was investigated using the Mongolian gerbil. Somatosensory evoked responses (SER) were measured in groups of animals before, during and after various periods of total cerebral ischemia induced by temporary bilateral common carotid artery (CCA) occlusion. The animals were anesthetized with sodium pentobarbital (70 mg/kg), secured in a stereotaxic frame, and platinized-platinum ball electrodes were placed upon the cortical surface. Responses to hind leg electrical stimulation were amplified using Grass P511H pre-amplifiers and processed with a Nicolet averaging computer. A scalar value depending upon first component latency and the differentiated response amplitude was calculated for each averaged response. After control (pre-occlusion) measurements, the carotid arteries were occluded and SER measurements continued for periods of occlusion from 10 to 80 minutes and reperfusion for up to 9 hours. During occlusion SER's were bilaterally depressed with total loss of short latency (<60 msec) components. The effect of duration of ischemia on return of SER's with reperfusion is shown in the accompanying figure. Each point represents the mean of SER return to 25% or 40% of control values in at least 8 hemispheres.



Recovery of SER's and the time course of this recovery seem dependent on duration of ischemia. Data on SER recovery, when related to post-occlusion blood flow and cortical neurotransmitter levels, indicate prolonged alteration in neurotransmission after transient ischemia. (This work was supported by NIH NS 09287-07)

392 DIFFERENTIATION BETWEEN CNS DRUGS IN MACACA MULATTA ON THE BASIS OF STABILITY OF EEG POWER SPECTRA. John E. Gehrmann, James W. Havstad* and Keith F. Killam, Jr.*, Dept. of Pharmacology, School of Medicine, U.C.D., Davis, CA 95616.

We have previously shown that a wide range of drugs acting on the CNS can be differentiated and grouped by analysis of the power spectrum of the EEG (Federation Proc. 35:2258-2263, 1976). These discriminations and groupings were based mainly on the analysis of average relative spectral power in selected frequency bands. This provided an initial basis for classification. However, visual inspection of temporal sequences of spectra indicated that the relative stability of the postdrug state might be of additional utility in further distinguishing the action of CNS agents, especially with regard to the benzodiazepines.

Power spectra of 34 min. segments of spontaneous EEG were calculated for 4 second contiguous epochs with a resolution of 0.25 Hz from 0.25 to 64 Hz. From each of these spectra, the mean power spectral density within each of 5 one-octave bands from 2 to 64 Hz was calculated. The resulting temporal fluctuations in spectral power for 34 min. segments for each of these bands was plotted for visual inspection of relative stability, and the mean and standard deviation also calculated. For further quantitative characterization of relative spectral stability, the power spectrum of each of the spectral band time functions was computed from 0.25 to 250 milliHz.

Meprobamate, glutethimide and amobarbital produced large increases in spectral power and a state which was more stable than predrug (especially at frequencies above 16 Hz). Phenmetrazine produced no noticeable effect for either measure. In contrast, the benzodiazepines: clorazepate, chlordiazepoxide, halazepam, and clonazepam produced moderate increases in total spectral power but showed a marked decrease in the stability of the postdrug state, especially in the 8-15.75 and 16-31.75 Hz bands. Additional distinctions among the benzodiazepines are reflected in the nature of the instabilities of the power spectrum, especially with regard to the periodicities of the fluctuations in time. These are measured by the spectral analysis of the spectral band time functions. These might correlate with the degree of behavioral depressions observed after drug administration.

(Supported by DEA contract #J-70-37.)

393 LOCALIZATION OF EXPERIMENTAL BRAIN STEM LESIONS IN CAT WITH MULTIMODALITY EVOKED POTENTIALS: CORRELATION WITH HUMAN HEAD INJURY EVOKED POTENTIAL DATA. Richard P. Greenberg and Donald P. Becker. Div. of Neurosurg., Medical College of Virginia, Richmond, VA 23298.

Multimodality evoked potentials, visual, auditory and somatosensory near field as well as auditory and somatosensory far field evoked potentials, were recorded from 51 comatose, severe head injury patients to locate areas of brain dysfunction not obvious from clinical evaluation (Greenberg, J. Neurosurg. 46, 1977). On the basis of this work in man we hypothesized that in head injury patients who had normal visual evoked potentials but absent or severely abnormal somatosensory and auditory near and far field evoked potentials brainstem dysfunction was marked and that cerebral hemispheric functions were relatively unaffected by the trauma.

To test this hypothesis experimentally the brainstem was completely transected (histologically confirmed) by radiofrequency lesions at the level of (1) the superior colliculi in 5 cats, (2) between the nuclei of the dorsal columns and the entrance of the eighth nerve in 5 cats and (3) the cervico-medullary junction in another 5 cats. Visual, somatosensory, near and far field (median nerve), auditory near and far field evoked potentials were recorded pre- and post-lesion in each animal.

Visual evoked potentials were unchanged from baseline regardless of the area of transection. Somatosensory potentials at approximately 4 msec, 6 msec, and 8 msec persisted after the superior colliculi transection while potentials beyond these latencies were abolished. With transection just caudal to the eighth nerve only potentials at 4 msec and 6 msec persisted. Following cervico-medullary transection all somatosensory potentials were extinguished. Auditory near field potentials were abolished after superior colliculi transection but not after transection caudal to the eighth nerve or at the cervico-medullary junction while auditory far field potentials persisted after all three transections.

These experiments suggest that multimodality evoked potentials are useful electrophysiologic tests with which to diagnose dysfunction of the human brainstem in vivo as they accurately reflect not just brainstem dysfunction but regional stem dysfunction allowing precise localization of injury in this area following head trauma. (Supported in part by N.I.H. grant NS12587 and a Southern Medical Association Training Grant).

394 EFFECTS OF ATTENTION AND STIMULUS SEPARATION ON THE AVERAGED VISUALLY EVOKED RESPONSE IN MAN. STEPHEN R. HARRIS, Psychological Consultant, 202 1/2 W. Washington Avenue, Jonesboro, Ark. 72401.

Averaged evoked cortical potentials (EPs) were investigated as a function of the attentional state and the subjects' (Ss') capacity to differentiate appropriate stimuli. Four adult human Ss viewed a small diffuse light stimuli (1° in diameter) in four positions (1°, 5°, 10° and 20° from the fovea). The stimulus to be attended was presented on one side of the display, and the nonattended stimulus was presented on the other side of the display at the same distance from the fovea. This left the separation between attended and nonattended stimuli either 2°, 10°, 20°, or 40°. The amplitude of the EPs was measured at the first major trough to peak deflection, the surface positive occurred between 150 and 200 msec following stimulation, while the surface negative peak occurred between 200 and 250 msec following stimulation. The data were quantified as percent difference in amplitude between attended and nonattended stimuli at each position. Analysis of variance indicated that the position of the stimuli was significant at the 0.025 level. It was concluded that attention may be studied effectively only when stimuli to be discriminated are presented to separate physiological systems or subsystems.

395 RELATIONS BETWEEN CORTICAL AND THALAMIC SOMATOSENSORY EVOKED POTENTIALS AT DIFFERENT FREQUENCIES OF STIMULATION. J.W. Hutchison* and J.A. Kusske. Neurosurgery Section, VA Hospital Long Beach and Div. of Neurological Surgery, UCI, Irvine, CA 92717

Evoked potentials and associated neuronal activity, induced by repetitive stimulation of the sciatic nerve with brief electrical pulses were recorded simultaneously from the somatosensory cortex, the ventroposterolateral nucleus and centre median nucleus, in cats under gallamine. The early component of the response, extending from the stimulus to 500 msec is of much higher amplitude than the late component, extending beyond the first 500 msec up to 2500 msec after the stimulus. For this reason the early and late components were analyzed separately at different magnifications. Averaging of the gross potential, post stimulus histograms of neuronal activity and autocorrelation studies show that, both in the early as well as in the late part of the response, the amplitude of the gross potentials, their rhythmicities and the probabilities of neuronal discharge with respect to time reach maximum values at specific frequencies of stimulation in the range of 0.25 to 12 pulses/sec. Relations between gross potentials and neuronal discharge within the cortex reach their highest cross correlation levels at specific frequencies of stimulation within the same range. Similarly the relations between gross potentials in the cortex and gross potentials in the thalamus, or between gross potentials in the two thalamic locations show their highest levels of correlation at specific frequencies of stimulation. These results suggest that the development of the somatosensory evoked potential and the associated neuronal response is related to the activity of cortical, thalamic and cortico-thalamic pathways which are most effectively "driven" at specific frequencies of stimulation.

396 CHANGES IN HEART RATE, RESPIRATION, AND EMG ASSOCIATED WITH OPERANT CONDITIONING OF 12-14 HZ SENSORIMOTOR ACTIVITY IN HUMANS. William J. Jackson, June O. Kearns*, Arden V. Nelson*, Dept. of Physiology, Medical College of Georgia, Augusta, Ga. 30902.

The neuroelectric activity of sensorimotor cortex has been controlled by operant conditioning or "feedback" techniques in a number of species, but most often in cats. The cat shows prominent trains of 12-14 Hz activity over the sigmoid (sensorimotor) gyri. Following operant conditioned enhancement of this "sensorimotor rhythm" (SMR), cats adopt a characteristic motionless posture and show decreases in muscle tone, heart rate, and rate of respiration. The clinical relevance of these findings came to notice upon the findings by Sterman and colleagues (Sterman and Friar, Electroencheph. Clin. Neurophysiol. 33:89-95 1972) that operant conditioning of 11-13 Hz sensorimotor activity reduced the frequency and severity of seizures as it had in cats. It is unknown whether there is a normal 12-14 Hz sensorimotor rhythm in humans. In humans, the 9 Hz rhythm "en arceau" or "wicket rhythm" is often presented as analogous to the 12-14 Hz rhythm in cats. However, it is also possible to record prominent trains of 12-14 Hz activity over the sensorimotor area in humans. We compared heart rate, rate of respiration, and submental EMG during alternating 5 minute periods during which the subjects first attempted to generate maximum 12-14 Hz activity and then later during 5 minute control periods. In contrast to the results of experiments using cats as subjects, we found that feedback enhanced 12-14 Hz activity over the sensorimotor strip was associated with increases in heart rate, increased amplitude of submental EMG, but decreased rate of respiration. The optimal site for recording 12-14 Hz activity was approximately 1 cm. posterior to Cz, C3, and C4. Recordings were between Cz-C3 and Cz-C4. It was possible to independently enhance the 12-14 Hz activity in both hemispheres, but somatomotor and visceromotor changes were equivalent regardless of which hemisphere was showing maximum 12-14 Hz rhythms. These results suggest that human 12-14 Hz sensorimotor activity is not associated with the same functions as 12-14 Hz sensorimotor activity in cats.

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397 SOME EARLY COMPONENTS OF THE HUMAN MEDIAN NERVE EVOKED RESPONSE. M. Kalichman and P. Raudzens*. Dept. Pharmacology, Univ. of Toronto, Toronto, Ont. M5S 1A8 (Canada).

The human median nerve evoked response to electrical stimulation has recently been described in great detail by Goff et al (1977). This response was described as having 20 components between 13 and 513 msec. The latencies of these components may eventually provide a valuable tool for non-invasive quantification of central nervous system (CNS) drug levels; diagnosis of CNS disorders; and monitoring of CNS activity.

In order to exploit the evoked response for these possible advantages, 2 initial steps were required. First, a specific part of the evoked response had to be chosen for optimizing drug sensitivity, reproducibility, and ease of recording. Based on these criteria, it was decided to use the interval 20-45 msec. after the stimulus. Secondly, analysis methods were selected for optimal reduction of non-stationary sources of evoked response variation (e.g. subject movement, electrical artifact, shifts in evoked response latencies). Two methods were chosen to meet this criterion. The first, adaptive filtering (Woody, 1967), will ideally align variable latency signals before averaging. The 2nd method was developed to sort out anomalous evoked responses according to cross-correlation coefficient magnitudes derived from the adaptive filter.

The median nerve response was recorded from 8 different subjects in 18 experimental runs. Recordings were T5-Fp1 (T6-Fp2 for dominant hand stimulation of left-handed subjects). Each experimental run resulted in 20 sets of 100 responses. The 2000 stimuli were 0.5 msec. monophasic pulses (5/sec.) with sufficient intensity to elicit a thumb twitch. Various versions of the 2 analysis methods were used on the sets of data (each stored as 10 averages of 10 responses). With each analysis method, 20 estimates were made of P25-N35 latency or P30-N35 latency. These groups of 20 numbers were each analysed for variability as a characterization of that particular analysis method.

Results:

1. For the above conditions, neither method resulted in improvements over averaging.
2. It has been suggested that not all individuals will present distinct P25 and P30 components (Giblin, 1964). This work suggests that this is not all-or-none between subjects

1. Giblin DR Annals of the New York Academy of Sciences 112:93-142, 1964.
2. Goff GD, Matsumiya Y, Allison T, Goff WR Electroencephalography and Clinical Neurophysiology 42:57-76, 1977.
3. Woody CD Medical and Biological Engineering 5:539-553, 1967.

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398 THE EFFECTS OF STIMULUS PATTERNING ON EVOKED POTENTIALS. Zaven S. Khachaturian, Richard R. Bishop*, Kurt L. Reisler*, Dept. Psychiatry, Univ. Pgh. Sch. Med., Pgh., PA 15261, and Dept. Elec. Eng., Penn State Univ., Univ. Park, PA 15802.

In an earlier study we observed that the waveform of evoked potentials (EPs) elicited by habituated stimuli were significantly influenced by the specific patterning of these stimuli. In order to study the influence of "expectancy" as a possible explanation, we investigated this phenomenon further in four chronically implanted cats. During 3-hour daily sessions for five days, the animals were habituated to light and click stimuli alternating at the rate of one/second. Subsequently, one of these stimuli (e.g., light) was presented in six different patterns against a background of continuous presentation of other stimuli (e.g., click) at the rate of one per two seconds. The distinguishing characteristics of the patterns were the number of stimuli (2^0, 2^1, 2^2, 2^3, 2^4, 2^5) presented in a train. During testing each of the six patterns was repeated 25 times in a randomized order at varied intervals. After 150 trials the stimulus modalities were reversed so that the formerly background repetitive stimulus became the "patterned" stimulus and vice versa, and the same procedure was repeated for another 150 trials. The EPs elicited by both the "patterned" stimuli as well as the background "non-patterned" stimuli recorded during the trial were averaged (N=25). The averaging was carried out so that the fidelity of the temporal order of the EPs was maintained, i.e., the 1st, 2nd, 3rd, nth EPs were averaged separately. The results indicated that in most structures the waveform of EPs are markedly influenced by the patterning of a physically constant stimulus. Along the primary sensory pathways these effects were particularly noteable in the EPs elicited by inappropriate modality stimuli e.g. auditory EPs recorded from visual cortex or visual EPs in MGN. These changes were complex, including component morphology, latency and amplitude. Unlike the human EP studies these changes were not limited to the late components (P300) but were present at earlier latencies.

These results indicate that late component changes observed in human scalp EP usually associated with complex psychological processes, have their origins in subcortical structures and may have more parsimonious neurophysiological explanations.

399 THE SPATIAL AND TEMPORAL PATTERN OF ALVEAR TRACT EVOKED POTENTIAL AND CURRENT FIELD IN THE HIPPOCAMPUS OF ANESTHETIZED RATS. L. Stan Leung. Dept. Physiol.-Anat., Univ. Calif., Berkeley, CA 94720

The alvear tract in the dorsal hippocampal CA1 region of anesthetized rats was stimulated electrically and the averaged evoked potential (AEP) field was mapped in the coronal, horizontal and sagittal planes. A clear topographical mapping was shown between dorsal CA1 and the alvear tract. From the depth AEP, the current vector field and current source-sink density maps in different planes, at least three events can be distinguished. The first event was the action potential invading the pyramidal cell bodies. The second event overlapped the first one spatially and temporally, and was marked by the increase of current source density at around the pyramidal cell layer. The third event was long-lasting (up to 200 msec) and was characterized by sources above and sinks below the pyramidal cell layer. Simultaneous post-stimulus time histograms showed pyramidal cells firing at the first and beginning of the second event. Inhibition, if any activity, was observed during much of the third event. These results are discussed in light of our current knowledge of hippocampal functional organization. Supported by MH06686.

400 SPATIO-TEMPORAL INTERACTIONS IN THE VISUALLY EVOKED POTENTIALS OF HUMAN STRABISMIC AND ANISOMETROPIC AMBLYOPES. Dennis M. Levi* and Ronald S. Harwerth* (SPON: S. J. Cool). University of Houston, College of Optometry, Houston, TX 77004.

Steady-state visual evoked potentials to the appearance/disappearance of sinusoidal grating patterns were recorded at temporal frequencies of 4Hz to 32Hz in amblyopic and non-amblyopic subjects. The averaged evoked potentials were evaluated via Fourier analysis. For a given spatial frequency, the responses of the non-amblyopic eyes are characterized by a log-linear relationship between the evoked potential amplitude and the grating contrast, over a range of about 1 log unit above threshold. The contrast responses of the amblyopic eyes show a lower slope and a higher saturation point than the non-amblyopic eyes. Additionally, the data show that the responses of the amblyopic eyes are reduced in amplitude, across the spatial frequency spectrum, with the differences between the two eyes increasing as a function of spatial frequency. Spatial tuning also varies as a function of the temporal frequency of the stimulus. Differences in spatio-temporal properties of the amblyopic and non-amblyopic eyes are considered in relation to neurophysiological studies of amblyopia in cats and monkeys.

401 POWER SPECTRUM ANALYSIS OF EEG AND AUTONOMIC CORRELATES OF TONE-INDUCED AWAKENINGS FROM SLEEP. Arnold Lidsky, E. A. Sersen,* J. Clausen*, D. Anderson*. N.Y. Institute for Basic Research, Staten Island, N.Y. 10314

Following four nights of uninterrupted sleep, during which vertex EEG, EOG, and autonomic functions were monitored, 10 young adult humans were stimulated on two consecutive nights by tones of increasing intensity until a key press response was performed. Twelve awakenings, in addition to pre- and post-sleep thresholds, were obtained on each night, with stimulation commencing early in the appearance of each of five sleep stages (1-4 plus REM). Percentages of sleep stages did not differ significantly between awakening and uninterrupted nights, while heart periods increased significantly on awakening nights, with stages retaining the same rank orders; i.e. longest periods for stage 2, shortest for awake and slow wave stages (3 & 4). Awakening reaction times were shortest for stages 1 and 2, followed by REM, and longest for stages 3 and 4.

The EEG power spectra were obtained by Fourier analyses of the frequency range of 1-32 cycles per second, with results summarized over six clinically relevant bands. Analyses of variance for each frequency band, including EEG data from prestimulation through five seconds postawakening latency in all stages, indicated significant differences between stages, up to 14 cycles per second. Stages 3 and 4 showed their peak power an 1-2 cycle/sec, and greater power than other stages for the delta, theta, and alpha ranges. Stages 1 & 2 had their peak powers in the theta range, while REM power declined monotonically with increasing frequency bands. In addition to sex effects and interactions, EEG power changes as a function of temporal proximity to initial tones and awakening latencies are of particular note. Relative to pre-stimulation segments of each sleep stage, power increased consistently with the onset of auditory stimulation, and generally decreased at least five seconds before awakening. While these events were consistent for the 1-12 cycles per second bands, the magnitudes of the changes increased from the alpha to theta to delta ranges. Furthermore, the latencies of power increments with stimulation (and decrements near awakenings) were inversely related to frequency band category; i.e. largest and earliest rises in the 1-2 cycle per second band. The meaning of EEG Power is discussed in relation to brain functional integrity.

402 EVOKED POTENTIAL CORRELATES OF DELAYED MATCHING IN ADULTS AND CHILDREN. Eileen B. Maisel* and Robert W. Thatcher (SPON: E. R. John). Dept. of Psych., N.Y.U. Sch. Med., New York, N.Y. 10016

Adults and children were tested in a delayed matching-to-sample task using upper and lower case letters of the alphabet. On each trial, a variable number of random dot patterns (control displays) were followed by a letter (standard) and more random dot patterns (inter-information-interval displays, ITIs). A comparison letter was presented last. Comparison letters matched or mismatched the standard. On match trials, the standard and comparison were either physically identical (both upper or lower case letters), or nominally identical (one upper and one lower case letter).

All displays were computer generated and presented in counterbalanced order at a repetition frequency of 1.1/sec. All stimuli were equated for intensity and visual angle and were 20 msec in duration. Participants were instructed to press one lever if the comparison display had the same name as the standard and another lever if it had a different name. Evoked potentials elicited by the displays were recorded from 11 monopolar derivations and a transorbital eye lead. In both adults and children EP waveforms elicited by comparison letters which matched the standard differed from EPs elicited by identical letters which mismatched the standard. In addition, there were differences between nominal identity and physical identity matches and between control and ITI random dot patterns. These effects were observed primarily in the late positive components of the EP.

403 HUMAN EVOKED RESPONSES TO COMPLEX VISUAL STIMULI. H.J. Neville*, E. Snyder, D.L. Woods*, C. Schulman-Galambos and R. Galambos. Dept. Neurosci., UCSD, La Jolla, Calif. 92093 (SPON: R. Galambos.)

This study was designed to see if late components of the human visual evoked response (VEP) to complex visual stimuli show reliable changes with cognitive variables in a no-task situation.

Young adults viewed equal numbers of colorful slides and flashes randomly intermixed. Slides showed pictures of people, places, etc. At the end of the session (128 slides) Ss rated each slide on a scale from 1-7 for "interest" and "surprise", and reported whether they had recognized the person or place in the picture when they first saw it.

VEPs were recorded from 3 midline and 1 ocular site, all referenced to the right mastoid. For each S VEPs were averaged separately for interest and surprise ratings and according to whether the person or place was recognized.

Late VEP components showed large and consistent variations depending on Ss reports of surprise, interest and recognition. The amplitude of P3 (400 msec) was more than twice as large for stimuli rated high than for those rated low on surprise. Slides which rated medium on surprise resulted in P3's of intermediate amplitude. The amplitude of N3 (500msec) also showed a monotonic relation with ratings of surprise: it was more than 10 times larger in VEPs to pictures rated low than high on surprise. While less robust, VEPs for high, medium, and low interest showed similar variations.

Slides "recognized" evoked P3's which were on the average 4 times larger than similar, unrecognized slides; each of 7 subjects tested to date has shown this effect. All VEP variations observed were largest and most consistent at frontal, rather than central or parietal scalp sites.

The fact that late components of the VEP show large and reliable changes with certain cognitive variables in a no-task situation suggests such paradigms may find useful application in clinical populations.

404 VER NONLINEARITIES WITH EQUAL-LUMINANCE CHROMATIC STIMULI. Gregory Phillips*, John Trimble. Depts. of Ophthalmology and Theoretical Biology/Biophysics, Univ. Chicago, Chicago, IL. 60637

Recent psychophysical and electrophysiological studies support the idea of chromatic and achromatic channels within the visual system. Furthermore, it appears as though these channels may have different temporal dynamic characteristics.

Thus far, few attempts have been made to study these channels using the visual evoked response (VER). Regan (Vis. Res.,13,1933, 1973) reported a VER correlate of stimulus wavelength which appeared to be qualitatively different from that of stimulus luminance. Van Hoek (Vis. Res.,16,205,1976) also provides VER data suggesting nonlinear interactions between color and luminance signals.

In an attempt to further clarify the temporal dynamic characteristics of chromatic and achromatic channels, we have applied the technique of white-noise analysis to VERs produced by pure chromatic stimuli.

Light from a red (660 nm) and green (565 nm) light-emitting diode was combined in a two-channel Maxwellian optical system. The subjective luminances of the LEDs were equated using heterochromatic flicker photometry. Keeping the mean luminances of the LEDs constant at these values, their intensities were modulated in counterphase by band-limited Gaussian noise. The first- and second-order Wiener kernels were then calculated for the VER to this stimulus using the fast Fourier transform.

For comparison purposes, kernels were also calculated for the red and green stimuli presented individually with the same mean luminance and dynamic range.

Kernels obtained for pure chromatic stimuli indicate that this channel has a lower bandwidth and significantly less nonlinear interaction than the achromatic channel. This finding is discussed in light of a model describing the temporal dynamics of VER mechanisms.

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405 EFFECTS OF MATERNAL PROTEIN DEFICIENCY UPON THE DEVELOPMENT OF AUDITORY BRAINSTEM RESPONSES IN RATS. Robert G. Plantz*, Don L. Jewett, and John S. Williston. Dept. Ortho. Surg., Sch. Med., UCSF, San Francisco, CA 94143 (SPON: R. H. Steinberg)

Two groups of timed-pregnant Sprague-Dawley rats were placed on either 8% or 24% protein diets on their fifteen day of pregnancy and continued on the diet after delivery. Four litters from each group were cross-fostered and the pups tested for their auditory brainstem evoked responses 8 times beginning at 16 days of age and continuing until day 43. Animals were anaesthetized with gaseous halothane and unilaterally presented with 60 db SL clicks. Signals were averaged from needle electrodes inserted into dorsal midline scalp and just below the pinna of the stimulated ear. Mean peak latencies for the first four waves were compared between groups using the Wilcoxson sum of ranks test. On days 16, 18, and 20 all four waves had significantly longer latencies in the undernourished groups than the controls ($P<.002$ for all waves). This difference decreased until day 43 when only wave IV was significantly slower.

An examination of other variables revealed an inverse correlation between body size and wave latency in the deprived group but not the control group. This suggests that development of each of the deprived group was limited by the amount of nourishment each received while the control group received enough nourishment to ensure normal development and also to build up body fat. An inverse correlation between body temperature and wave latencies was significant and suggested that the undernourished pups, which had lower body temperatures under anesthesia during recording than did the controls, may have had slower latencies because of their cooler temperatures. A second study was run with 7 new litters (4-24% and 3-8%) of animals, using the same procedure as before with the exception that body temperatures were held at 36°C for both groups using a water heated pad. These pups were run on their seventeenth day only and showed that while differences between groups were slightly smaller, they still differed significantly for all four waves (<0.05 for wave I and <0.01 for all other waves).

For reasons enumerated in a previous study (Jewett and Romano Br. Res. 36:101, 1972) these data cannot answer the question of whether the locus of the site of the effect occurs at the recep tor, in the central nervous system, or both. They do however indicate that in the rat neonate, undernutrition does effect early sensory pathways in a way that can be detected by far field evoked response averaging.

406 FOCAL RADIATION AND THE BLOOD BRAIN BARRIER. Michael P. Remler. Dept. of Neurology, State Univ. at Stony Brook, NY 11794.

Adult cats with chronic epidural electrodes were given between 250,000 and 1,000,000 U. of parenteral Penicillin G before and in test doses for one week after focal cortical irradiation of 4000 Rads. EEG's recorded during penicillin administration showed focal epileptiform spikes from irradiated area (Conf. Neurol. 35:50, 1973). Histological examination showed no significant changes. This is interpreted as the radiation causes physiologic disruption of the Blood Brain Barrier without fixed anatomical distruction. The penicillin crosses the Blood Brain Barrier only in the irradiated area and causes the epileptic discharge. This supports other evidences that radiation may affect the Blood Brain Barrier at doses below the histologically lethal or visable range.

Supported by Veterans Administration General Research Fund.

407 SENSORY FIELD POTENTIALS AS A TOOL TO MONITOR LEVEL OF KETAMINE INDUCED ANESTHESIA. B.M. Rigor* and N. Dafny (SPON: W.A. Weems). Departments of Anesthesia and Neurobiology, The University of Texas Medical School, Houston, Texas 77030.

The effects of 10 incremental doses of ketamine on photic and acoustic evoked responses were determined in five brain sites in freely behaving rats. Permanent semimicroelectrodes (60 in diameter) were implanted stereotaxically under pentobarbital anesthesia within the inferior colliculus (IC) and lateral geniculate body (Lgb), the primary relay sites for acoustic and photic input, respectively. Three additional electrodes were placed in the mesencephalic reticular formation (RF), in the ventromedial hypothalamus (VMH) and within the somatosensory cortex (SC). Following recovery from surgery (6-8 days) the averaged acoustic and photic field potentials following 32 repetitive stimuli were averaged as one set using a 1070 NIC minicomputer. Four sets were recorded at 10 minute intervals before and after each injection. Ten doses of ketamine were studied: 1; 5; 10; 20; 40; 60; 80; 100; 120 and 140mg/kg i.p. Each animal was tested twice with one week interval. In each experiment only 5 randomly selected incremental different doses were used. The dose which induced surgical anesthesia was found to be 80mg/kg i.p. Differences in the number of ketamine-induced changes in sensory field potentials were observed between the photic and acoustic input, where the acoustic evoked responses were more sensitive and exhibited dose-dependent responses, particularly in the SC. The lower subanesthetic doses of ketamine (<80mg/kg) induced depression of the responses while higher doses (100-140mg/kg) induced increases in the response amplitudes, shifted the amplitude to the right (longer latency) and prolonged the duration of the responses. However, the responses from the sensory relay nuclei were affected by the initial dose of 1mg/kg and remained about constant following the remaining doses. The RF and VMH were affected differently by ketamine. In conclusion, the acoustic evoked responses from the SC can be used as an indication of the level of ketamine anesthesia.

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408 EVOKED POTENTIAL MEASUREMENTS SUGGESTING LATERALIZED HEMISPHERIC DYSFUNCTION IN CHRONIC SCHIZOPHRENICS. Richard A. Roemer and Charles Shagass*. Eastern Pennsylvania Psychiatric Institute and Temple University Health Sciences Center, Philadelphia PA 19129.

Evoked potentials (EPs) to checkerboard pattern-onset (VEPs), auditory clicks (AEPs), and right and left median nerve (SEP) were obtained from 25 chronic schizophrenic patients and equal groups of nonpsychotic inpatients and nonpatient controls (matched for age and sex). Measurements of EP amplitude and wave-shape variability over time were computed. These were analyzed to determine (a) the extent to which the groups differed with respect to these measures on individual leads for each stimulus type; (b) group differences in measures of hemispheric lateralization, based upon differences between EPs from homologous leads over the two hemispheres for each stimulus type.

EPs were obtained from 14 scalp and one EOG lead. Amplitudes for two epochs (50-150, 151-450 msec for VEP, or 15-100 and 101-450 for AEP and SEP) post-stimulus were computed by Shagass' average deviation method. Variability for these epochs was determined by calculating the correlation between corresponding data points for four consecutive averages; these r's were transformed to Zr for statistical analyses.

Multivariate profile analyses, on the Zr after adjustment for covariance with amplitude and EOG, revealed a number of significant differences. The differences with VEPs and AEPs were due to increased left hemisphere variability in the temporal and occipital areas of chronic schizophrenics. The SEP differences revealed a more stable early response (15-100 msec) and a more variable late response (101-450 msec) at the central leads. None of the differences were related to handedness. The results confirm several earlier reports of greater EP variability in chronic schizophrenics, and demonstrate one kind of relative left hemisphere dysfunction in these patients.

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409 ELECTROPHYSIOLOGIC STUDIES OF MULTIPLE SCLEROSIS. V.S. Rossiter, M.L. Tracy*, M.E. Seybold, J.A. Aalbers* and J.J. Stockard. Dept. Neurosciences, UCSD Medical School, La Jolla, CA 92093.

A battery of five electrophysiological tests were performed on 20 neurologic patients with known or suspected multiple sclerosis (MS). Diagnostic classification of possible, probable or definite MS was determined according to McAlpine's criteria (McAlpine et al Multiple Sclerosis: A Reappraisal, 1972). Tests included Brainstem Auditory Evoked Responses (BAER's), Pattern Shift and Diffuse Photic Visual Evoked Responses (PS VER's and DP VER's, respectively), Blink Reflexes and Electrooculograms (EOG's). Sampling was biased to the extent that patient selection was based primarily on history or presence of neuro-ophthalmologic abnormalities, and 19 of 20 patients had clinical evidence of optic neuritis and/or internuclear ophthalmoplegia (INO).

Results are presented in the table below. Eight patients were given a diagnosis of possible MS, 4 patients were probable MS candidates and 8 patients were given a diagnosis of definite MS. Only one patient (possible MS) had normal results on all tests. Patients with a diagnosis of probable or definite MS had abnormalities on two or more tests. PS VER's were superior to DP VER's in assessing lesions of the optic tract, and EOG's were effective in assessing INO's. BAER's were most effective in detecting "silent" lesions in the brainstem auditory pathway. Blink reflexes were abnormal in only two patients and were least effective in confirming the diagnosis of MS. Our findings suggest that a battery of electrophysiologic tests, including BAER's, PS VER's and EOG's can be a useful adjunct in the diagnosis of multiple sclerosis.

		Abnormal Test Results				
		BAER's	VER's		EOG	BLINK
Diagnosis			PS	DP		
Definite MS (n=8)		6	2	3	7	2
Probable MS (n=4)		3	1	0	4	0
Possible MS (n=8)		1	5	2	5	0
TOTAL:	20	10	8	5	16	2

410 AN ELECTROENCEPHALOGRAPHIC RESPONSE SPECIFIC TO DBA/2 MICE. Lawrence J. Ryan* and Seth K. Sharpless. Dept. Psych., Univ. Colo., Boulder, CO 80309.

We have identified bursts of regular, high amplitude spikes in the cortical EEG of adult (60-70 day) DBA/2Ibg mice which are not associated with overt seizures or myoclonic jerks. These episodes are unique enough that DBA mice may be distinguished from mice of the other strains tested by visual examination of their EEGs. The spikes have a frequency of 6.9-7.8 Hz with each episode lasting 0.3-5.0 seconds. Of the 15 DBA mice of this age tested, all displayed at least 9 episodes with a maximum of 52 and an average of 20.3 during a thirty minute recording session. Injection of 30 mg/kg Metrazol, ip, increases the episode's frequency of occurrence. The spike episodes were observed in both restrained and freely-moving mice. Body movements occurred during only 3% of the episodes, but were recorded preceding or following (±5sec.) over 42% of the bursts.

During an episode the spikes are bilaterally symmetrical, occur simultaneously and with the same polarity frontally and posteriorly, and reverse 3mm lateral to the midline. Single spikes occasionally appeared frontally but seldom posteriorly.

To test if these episodes relate to DBA's seizure susceptibility during early life, a preliminary developmental study was begun. Initial results indicate that the episodes are of lower amplitude and are less well organized in 21 and 26 day animals but have a more adult appearance at 32 and 37 days.

Three additional strains were tested to determine the generality of the phenomenon. These spike episodes were not observed in C57BL/6Ibg (N=11) or BALB/c(N=14) mice. Similar episodes which occurred much less often (2.56 episodes/30 minutes), with a lower amplitude and a shorter duration were identified in 6 of 9 C3H mice tested.

DBA mice, then, display a distinct cortical EEG pattern which does not occur in two strains (BALB and C57) and occurs less often and less distinctly in another (C3H). The response's amplitude (5-10X ongoing activity) and frequent occurrence make the spike episodes a conspicuous cortical event warranting further examination.

This research was supported in part by the Council for Tobacco Research Grant 1076.

411 BRAINSTEM EVOKED RESPONSE AUDIOMETRY IN NEWBORN HEARING SCREENING. C. Schulman-Galambos and R. Galambos, Dept. Neurosciences, U.C.S.D., La Jolla, Calif. 92093

Brainstem evoked response audiometry (BERA) has been used as an auditory screening procedure in three groups of newborn infants. Group I consisted of 220 normal term babies tested within 72 hours of birth; no hearing abnormalities were uncovered (386 ears) and their threshold response (for clicks) lay between 10 and 20 dB HL (re adult). Group II consisted of 75 newborns treated in an intensive care unit for 1-14 weeks; 4 were found to have severe sensorineural hearing loss (7 ears) at the time of discharge. Group III consisted of a subgroup (35) of 325 infants one year or older who had previously been discharged from the same intensive care unit; of these an additional 4 showed severe sensorineural hearing loss. All abnormalities identified by BERA were subsequently confirmed by conventional audiometric measures. The estimate of an incidence of severe hearing loss in 1 of 50 babies who required intensive care in the neonatal period calls for careful testing of this population .

412 EFFECTS OF REPEATED ADMINISTRATION OF d-AMPHETAMINE ON THE OCCURANCE OF THE SENSORIMOTOR RHYTHM (12-14 Hz.) IN CORTICAL AND LIMBIC AREAS OF THE CAT. J. Marc Simard*, Calvin C. Turbes, Gerald T. Schneider* (SPON: J. F. Masken). Creighton Univ., Omaha, NE 68178.

The Sensorimotor Rhythm (SMR), recorded from the sigmoid gyrus the septum and the amygdala (all ipsilateral) was studied by spectral analysis, prior to and following repeated administrations of d-amphetamine sulfate (d-A.) (2.5 mg/kg, I.P., each day for 3 days). Ongoing power spectral arrays before administration of d-A., during wakefulness, indicate that power in the 12-14 Hz. band is greatest in the sigmoid gyrus, least in the amygdala and of intermediate value in the septum. Cross-power spectra verify observations of the raw data that spindles of SMR activity often occur simultaneously in the 3 areas studied. Occurance of the rhythm is well correlated with behavioral freezing. During slow-wave sleep, the power relationship between the sigmoid gyrus and the septum reverses, when bursts of the septal rhythm may reach 400 μV. During these epochs, power in the SMR band in the amygdala generally increases.

After an initial injection of d-A., the cortical activity remains desynchronized, while the deep areas take on a slow-wave component correlated with respiration. After 3 days of daily administration of d-A., the slow-wave activity of the sigmoid gyrus is seen to alternate exclusively between a fast, low-voltage rhythm, and the 12-14 Hz. rhythm. The fast rhythm correlates behaviorally with movements or shifts in gaze, while the SMR rhythm occurs when a frozen or staring posture is assumed. Continuous trains of SMR activity of 60 sec or more are often observed seperated from each other by only 1-5 sec of desynchronized activity. Such high percentages of time spent in SMR activity are not observed before administration of d-A., or immediately preceeding the 3rd injection of d-A. The last observation would indicate that abstinence from food, due to d-A., is not the exclusive cause of the observed increase in SMR activity. Preliminary observations have also indicated that the onset and buildup of SMR trains occurs shortly after the animal is seen to freeze behaviorally.

(This work was supported by a Doctoral Dissertation Research Fellowship to JMS from the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction, U.S.A.)

413 SOMATOSENSORY EVOKED RESPONSE AND SENSORY THRESHOLD: DETECTION OF A PERIPHERAL EVENT. Hilton Stowell. Sensory Neurophysiol. Lab., CSH, Georgia 31062.

Event related brain potentials (ERBP), recorded on human scalp or superficial cortex, show ambiguous correlations with sensory thresholds. One subdural study of somatosensory evoked responses (SER) during thalamic nuclear and peripheral skin stimulation (Libet et al., ICS 253: 157-168, 1971) indicates that the primary positive of the SER, though necessary, is insufficient for an introspective, conscious recollection of sensation, and that backward masking of conscious sensation by direct cortical stimuli is effective up to 200 ms after the peripheral stimulus. I am now studying the scalp-recorded SER to subthreshold and threshold electrical pulse stimulation of digits, using a self-stimulation paradigm designed to optimize stability of attention, expectancy, and those task-related variables which are known to affect both sensory thresholds and the waveform of the 60-200 ms time-segment of ERBPs (Hillyard, PROC. NIMH-ERBP CONF. IV: 1-54, 1977). Subjects count the number of self-administered trials (32 per block) and report either the number or percentage of stimuli detected, together with open-ended descriptions of the spatiotemporal and qualitative aspects of sensation. In a control paradigm various percentages of supra-threshold stimuli are randomly injected by experimenter into a block of 32 no-stimulus trials. Under these conditions, the "vertex" positive wave (150-250 ms) is the only valid and reliable correlate of stimulus detection. However, the temporal relationship of averaged, transient (200-500 ms), 9-13 Hz periodicity to both SER primary positive and "vertex" positivity seems to covary with both stimulus parameters and subjective report of sensory quality and detectability.

It is important to note that these data refer to reports of recollected, conscious sensation and not to reaction times or forced-choices in a signal-detection paradigm.

414 EVOKED POTENTIAL CORRELATES OF LOGIC: NEGATION AND EQUIVALENCE.
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Seven adults were tested in a logic task. A given trial involved the presentation of a variable series of random dot stimuli then a letter (A,B,C, or D) followed by an operator sign (equals, =; not equal, ≠; or no operation, ⌐┘), followed by a second letter. The subjects were instructed to differentially move a two position lever depending on whether the statement formed by the operator and letters was "true" (e.g., A = A, A ≠ B), "false" (e.g., A ≠ A, A = B), or "no operation" (e.g., A ⌐┘ A, A ⌐┘ B). All combinations of letters and operators were presented and all conditions were counterbalanced across a session. The stimuli were computer generated, 20 msec in duration and presented at a repetition frequency of .66/sec. All of the displays were equated for intensity and visual angle (1.5°).

Evoked potentials (EPs) elicited by the displays were recorded from 11 scalp derivations and a transorbital eye lead. EPs elicited by letters in the "truth" condition were different than EPs elicited by identical second letters in the "false" condition. Likewise, EPs elicited by second letters in the "no operation" condition were different than EPs elicited by second letters in the "truth" and "false" conditions. Varimax factor analysis revealed a differential scalp topography for EPs elicited by controls, first letters, operators, and second letters. These analyses indicate extensive frontal lobe involvement.

415 USE OF A DISSOCIATIVE ANESTHETIC (CI 744) FOR UNCOUPLING COMPONENTS IN THE STUDY OF CAT BRAIN ELECTRICAL RHYTHMS.
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The dissociative anesthetic CI 744* is a mixture of the phencyclidine derivative tiletamine hydrochloride (CI 634*) and flupyrazapon (CI 716*). In doses of 8-30 mg/kg injected intramuscularly it produces a rapidly-induced (1-2 min), relatively short-acting (0.5-3hr) dose related anesthesia characterized by a cataleptoid electroencephalographic (EEG) pattern. Recordings obtained among multiple sites on the cortex by chronically implanted electrodes showed that CI 744 induces a high voltage synchronization of EEG waves (1-3 hz band) in neocortical areas. Stereotaxically placed deep electrodes recorded similar waves from the reticular thalamus. EEG signs of recovery from effects of the anesthetic include a rapid change (30-60 sec) from almost exclusively slow wave activity to desynchronization typical to that of awakening. Spindling is never provoked by the drug in the intact brain but in animals with electrolytic destruction of midbrain ascending reticular activating system, the spindling as well as slow wave activity is increased. However, recruiting produced by low frequency electrical stimulation of the reticular nuclei of the thalamus is depressed by CI 744 but it blocked neither spindling resulting from interruption of ascending reticular activating system nor spindles elicited by pentobarbital. This evidence supports the concept that recruiting is probably an electrophysiological correlate of several neural activities, of which the spindles found in barbiturate anesthesia and the early stages of sleep are just one. Whereas EEG desynchronization could be induced during pentobarbital anesthesia by strong nociceptive stimulation, electrical stimulation of pain afferents and by electrical activation of the midbrain reticular formation, under CI 744 EEG arousal was entirely absent during these stimuli even though the flexion reflex and a pupillary reaction to light were clearly present. On the contrary, in lightly anesthetized animals a decrease in frequency of the basic slow wave pattern was the only EEG detectable response to arousing stimuli. This work partially supported by CNPq and FAPESP.
* Parke-Davis and Co.

416 THE Q_{10} OF AUDITORY BRAINSTEM RESPONSES IN RATS UNDER HYPOTHERMIA.
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Changes in the latencies and heights of ABR (Auditory Brainstem Responses) in response to body cooling were studied in 38 Sprague Dawley rats ranging in age from 15 to 120 days. The animals were anesthetized with 2% gaseous halothane and presented 50μsec clicks at 10 to 80 clicks per sec, and at 10 to 60 db click intensities. Body temperatures were regulated by a temperature-controlled copper plate upon which the animals were placed. Signals were averaged by needle electrodes inserted into midline dorsal scalp at the interaural line and at the pinna of the stimulated ear.

Following a series of control recordings taken at 38°C the animal was cooled in stages and further records taken after body temperatures stabilized. Body temperature was monitored by thermocouples inserted into the ear canal of the nonstimulated ear (shown to be equivalent to intracranial brain temperature). Rectal temperatures were found to not accurately reflect temperature changes in the brain.

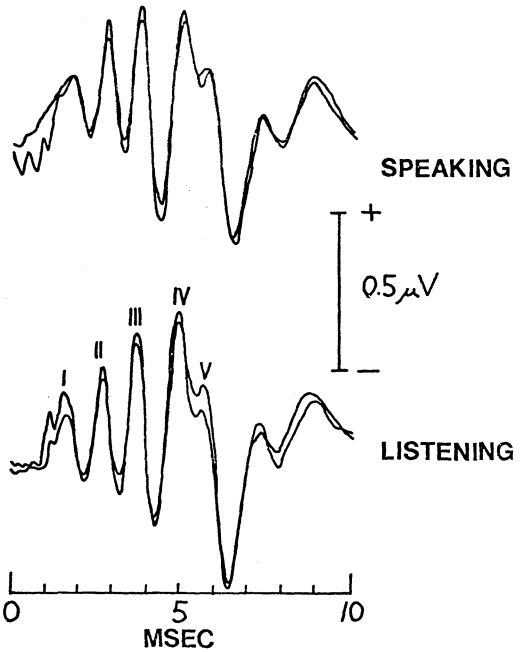
Although variability between animals was considerable (see ranges in Table I), there was very high repeatability in wave latencies and Q_{10} between runs taken at a constant temperature, high or low, in individual animals and changes in wave latency with cooling were very predictable within a single session. Changes in either repetition rate or intensity did not appear to interact with the effects of hypothermia. The large variability in these data suggest that it may not be possible to "correct" evoked response latencies for changes in temperature by the application of a single mean Q_{10} ratio obtained from a population, young or adult, despite the statistically significant change with age.

Table I. Q_{10} Values for Latencies of ABR Components at Different Ages.

Wave #	15-20 Days Old Median	(Range)	21-120 Days Old Median	(Range)	Wilcoxon's Sum of Ranks Test
I	1.87	(1.4-2.3)	1.63	(1.2-2.0)	P<.01
II	1.83	(1.5-2.4)	1.53	(1.4-1.8)	P<.002
III	1.88	(1.3-2.2)	1.57	(1.3-1.9)	P<.05
IV	1.57	(1.4-2.3)	1.55	(1.2-2.0)	P>.10

417 BRAINSTEM EVOKED RESPONSES DURING SPEECH PRODUCTION.
David L. Woods*, Robert Galambos and Steven A. Hillyard. Dept. Neurosci., UCSD, La Jolla, Calif. 92093.

We compared click-evoked brainstem responses (BERs) in subjects who were 1) producing speech and 2) listening passively to tape recordings of their own speech, to see if vocalization in man is accompanied by the same efferent inhibition of brainstem auditory relay nuclei as has been reported to occur in the bat (Suga, 1974). Two experiments were conducted, one where speech was fed back immediately to the speaker, and another where speech was fed back after a 200 msec. delay. In both experiments the BERs during speech production were indistinguishable from those during passive listening, suggesting that speech production does not engage special efferent inhibitory mechanisms in man.



EPILEPSY

418 THE INFLUENCE OF TRACE METAL CHELATION ON SEIZURE ACTIVITY IN THE SENEGALESE BABOON. M.C. Alley* and E.K. Killam*, Department of Pharmacology, School of Medicine; and G.L. Fisher*, (SPON: R.M. Joy). Radiobiology Laboratory, University of California, Davis, CA 95616.

Several clinical and experimental investigations have suggested a possible relationship between seizure activity and abnormal metabolism of copper and/or zinc. The finding of elevated titers of serum zinc in the seizure-prone baboon species, Papio papio, as compared to the non seizure-prone primate species, Papio cynocephalus and Macaca mulatta, led us to consider whether zinc status might be related to the higher degree of seizure proclivity in the former species. D-penicillamine, known to "selectively" chelate divalent metal cations which are present in excess, was found to exert significant anticonvulsant activity in several chronic phases of treatment. Daily oral doses of 80-120 mg/kg given over a four week period decreased the severity and spread of seizures induced by intermittent light stimulation (ILS). Lower doses of 40-80 mg/kg given over a nine week period were more effective than the shorter courses of high dose treatment, and were not accompanied by noticeable side effects. Moreover, azaribine, structurally unrelated to D-penicillamine, but known to induce "indirect chelation" of copper and zinc was found to exert a similar form of anticonvulsant activity in these baboons at daily oral doses of 25-50 mg/kg. Seizure latency was lengthened, while seizure duration and spread were progressively diminished or entirely blocked over consecutive weeks of treatment. Both agents promote a quieting effect upon behavior without signs of sedation. Blood and urine samples were collected during these studies and evaluated for trace metal content. The altered patterns of seizure response and serum trace metal titers observed during the courses of pharmacologic intervention will be discussed.

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419 INHIBITION IN PENICILLIN -INDUCED EPILEPTIC FOCI. T.E. Anderson* and L.T. Rutledge. Neuroscience Program, University of Michigan, Ann Arbor, Michigan 48109.

Though topical application of penicillin is a widely used experimental model of epilepsy, the neuronal mechanisms responsible for development of epileptiform activity remain poorly understood. It was reported that the evoked potential recorded from cat precruciate cortex, subsequent to pyramidal tract stimulation, was altered after application of penicillin to the cortical surface. The early surface negative component (SN-wave, associated with recurrent inhibition) decreased in amplitude over time, but it recovered upon removal of the penicillin. It was proposed that a decreased effectiveness of recurrent inhibition was the basis for epileptogenicity in a penicillin focus. To study the proposed loss of inhibition at the neuronal level, and to assess possible sites of penicillin action, foci were made in chloralose anesthetized cats. The effect of pyramidal tract, epicortical and foot pad stimulation upon spontaneous activity of extracellularly recorded neurons was calculated prior to and at intervals subsequent to application of penicillin to precruciate cortex. Only neurons yielding stable recordings throughout the transition from normal to epileptogenic cortex were counted. Cells were categorized as pyramidal tract (PT) or non-PT on the basis of antidromic activation following stimulation of the cerebral peduncle. For PT cells, inhibition observed in normal cortex was less effective subsequent to establishment of an active focus, regardless of the stimulus mode. However, excitability of bursting non-PT cells (B-NPT) increased subsequent to penicillin application, again irrespective of the stimulus modality responsible for the initial excitation. Further, 68% of the B-NPT cells exhibited an increase in firing in response to pyramidal tract stimulation which preceeded SN-wave peak amplitude, firing behavior consistent with their involvement in recurrent inhibitory pathways. These results demonstrate a loss of inhibitory effectiveness at the pyramidal cell level, as implied by SN-wave alteration. However, the loss of inhibition occurs simultaneously with an increased excitability of neurons which appear to be involved in inhibitory circuits. Thus, penicillin is probably not acting to reduce excitability of inhibitory interneurons or efficacy of synaptic inputs to those cells. Penicillin likely acts to increase apical dendritic membrane resistance. Applied topically it would selectively enhance the influence of the more superficially terminating excitatory inputs; it would also reduce the passive superficial current sink and hence, the surface negative wave.
(Supported by grants MH 14279 and NINCDS 04119)

420 STIMULATION OF THE CEREBELLAR SURFACE AND ITS EFFECT ON THE ACTIVITY IN PENICILLIN FOCI. Heinrich Bantli and James R. Bloedel. Dept. Neurosurg., Med. Sch., Univ. Minnesota, Minneapolis, MN 55455.

The effect of stimulating the cerebellar surface on the activity in acute penicillin foci (20,000 units) was investigated in a statistically controlled study. At the onset of an experiment, the cats were assigned randomly to either the experimental group (stimulating electrode placed over the vermis of the anterior lobe) or to the control group (only the dura opened over the posterior lobe). Following surgery, the animals in the experimental group were again randomly assigned to either the stimulated or unstimulated group. The cerebellar stimuli were capacitatively coupled pulses applied at a frequency of 10/sec with current densities of.25 ma/cm^2 and durations of .1 ms. The stimuli were applied with a bipolar electrode consisting of two platinum plates with a surface area of 7.6 mm^2 per plate. In order to determine the effectiveness of the stimuli to activate the cerebellar tissue, the cerebellar evoked response was recorded from the sensorimotor cortex prior to the injection of the penicillin. The statistical analysis indicated that any difference between the means of the total number of seizures per animal or total seizure time per animal of the control and experimental groups could have occurred by chance with high probability. But the distribution of the durations of the seizures was significantly affected by the cerebellar surface stimuli. In addition, the correlation of the amplitude of the cerebellar evoked response with the total number of seizures indicated that these two parameters did not vary independently, indicating that stimuli evoking large-amplitude responses over the sensorimotor cortex produced a decrease in the number of seizures in the same animal, whereas stimuli that evoked small-amplitude responses produced an increase in the total number of seizures with respect to the mean number per animal in the control group.

This work was supported by NIH Contract NS4 2332.

421 CORTICAL EXCITABILITY CYCLES FOLLOWING INTERICTAL "PENICILLIN SPIKES" IN CAT. F. M. Barken* and E. L. Gasteiger. Section of Physiology, Div. of Biology and N.Y.S. Vet. Col., Cornell U., Ithaca, NY 14853.

Triggering of topical penicillin-induced interictal spikes was accomplished by orthodromic activation of a cortical focus through stimulation of nVL of the thalamus in cats anesthetized with urethane. Condition-test stimuli delivered to nVL produced paired penicillin (PCN) spikes (surface positive-negative waves) only when the stimulus interval exceeded 150 msec. Facilitation of the positive wave on the order of 120 to 140% peaked at an interval of 250 msec. whereas maximum facilitation of 120 to 200% occurred at 300 to 350 msec. for the negative component. At intervals of 800 msec. and greater no facilitation of the test penicillin spike was observed. Facilitation was demonstrated in 10 out of 13 animals.

Single stimuli to nVL were delivered at varying intervals following spontaneous PCN spikes. Spikes could not be triggered with a delay less than 150 to 200 msec. following the spontaneous discharge. Facilitation of both the positive and negative waves of the nVL-triggered PCN spike has been observed at 250 and 350 msec. respectively, after the spontaneous spike in 6 out of 9 animals. Spontaneous and triggered PCN spikes appear to behave similarly with regard to their excitability cycles.

Different underlying mechanisms of electrogenesis are indicated by the differing latencies for maximum facilitation of the positive and negative PCN spike components. We interpret this facilitation as originating from interacting thalamocortical influences, since after-discharges are seen at the cortex 200 to 250 msec. after PCN spikes and arise from reverberatory circuits between thalamus and cortex as demonstrated by reversibly cooling nVL.

(Supported by Grant in Aid-of-Research from Natl. Chapter Sigma Xi)

422 EFFECTS OF EPILEPTIFORM DISCHARGES IN THE VISUAL CORTEX ON NEURONAL DEVELOPMENT IN THE LATERAL GENICULATE NUCLEUS AND SUPERIOR COLLICULUS OF YOUNG RABBITS. H. Dale Baumbach, K.L. Chow, Barry L. Gordon*, Dept. Neurol., Stanford Univ. Sch. Med., and David L. Glanzman*, Dept. Psychol., Stanford Univ., Stanford CA 94305.

The effects of an epileptogenic focus in the visual cortex on the development of receptive field properties of single cells in the dorsal Lateral Geniculate Nucleus (LGN) and Superior Colliculus (SC) were studied. 8 to 9 day old rabbits were chronically implanted with a stainless steel cannula on each hemisphere so as to rest on top of the dura overlying the VI area of the visual cortex. To create an epileptogenic focus, penicillin (200,000u/ml) was applied to the dural surface through one cannula twice daily beginning one day after implantation. Interictal spiking began 3-10 min following the initial application and continued for 6-12 hrs. As a control, penicillin (200,000u/ml) mixed in equal proportions with penicillinase was simultaneously applied through the second cannula. For the LGN study, penicillin applications were continued to 19-24 days of age and the animals were prepared for single unit recordings at 20-25 days of age. For the SC study, applications were continued to 21-26 days of age and animals prepared for recordings at 22-27 days of age. Both of these age ranges are well beyond the time at which previous work (Rapisardi, Chow, & Mathers, 1975; Fox, Chow & Kelly, 1976) has shown that receptive fields in each respective structure achieve adult-like characteristics. All drug applications were discontinued 24 hours prior to single unit recordings and no interictal spiking was present during the single unit recording sessions.

The development of receptive field properties of cells in the LGN ipsilateral to the epileptogenic focus was clearly disrupted. There was a significant increase in the proportion of cells which were either unresponsive or had indefinite receptive fields along with a significant decrease in cells with concentric receptive fields. The proportional distribution of receptive fields for cells sampled in the LGN ipsilateral to the control injections was normal. Preliminary data on the Superior Colliculus, on the other hand, indicates that its development is not affected by the presence of the epileptiform focus. All types of receptive fields were found in the SC ipsilateral to the epileptiform focus and the proportional distribution did not differ appreciably from normal.

The presence of cortical interictal events during early development appears, therefore, to disrupt normal development in Thalamic structures remote from but synaptically related to the focal area. (Supported by NIH grants EY00691 and NS12151 to K.L.C., NIH grants EY05176 to H.D.B. and NS07012 to B.L.G.)

423 INFLUENCE OF 5-HYDROXYTRYPTOPHAN (5-HTP) AND DIMETHYL TRYPTAMINE (DMT) ON ISCHEMIA-INDUCED SEIZURES IN THE GERBIL. James C.H. Chan*, K.M.A. Welch, Eva Chabi*, and T-P.F. Wang.* Dept. Neurol., Baylor Coll. Med., Houston, Tx. 77030.

Ischemia-induced seizures in the gerbil were studied as a model of epilepsy. The entire gamut of seizure activity, from myoclonic jerks, turning and running fits, to tonic-clonic fits, was observed in over 70% of gerbils studied after a 30 minute episode of cerebral ischemia was produced by bilateral common carotid occlusion followed by reperfusion. Gerbils were observed for seizure activity for up to 5 hours after the ischemic insult. Fifty percent of animals that seized died during a tonic fit. The onset of seizure activity was marked by running fits which appeared at fairly discrete, equally spaced time intervals, clustered around 1.00, 2.25 and 3.50 hours after cerebral reperfusion.

Previous studies had shown depletion of monoamines in ischemic brain of the gerbil (Zervas et al., Nature 247, 283, 1974; Welch et al., Proc. Amer. Soc. Neurochem. 6, 156, 1975). When we subsequently investigated the differences in brain monoamine levels between animals with cerebral ischemia alone and animals with ischemia that developed seizures, we found that catecholamines were significantly depleted in the cerebral cortex of the ischemia plus seizure group but not in the group with ischemia alone (Welch et al., Trans. Amer. Neurol. Assoc., in press, 1977). Serotonin was decreased in both groups of animals. This raised the possibility that (1) decrease in catecholamine levels was a secondary effect of seizure activity, and (2) since pharmacological depletion or elevation of brain serotonin respectively facilitates or decreases seizure threshold in other epilepsy models (Maynert et al., in Freidlander, Adv. Neurol., Vol. 13, Raven Press, N.Y., 1975), the decrease of serotonin common to both the ischemia and ischemia plus seizure groups was a factor predisposing to the development of seizures in the latter. Serotonergic influence on seizure activity was therefore examined in the gerbil model by pharmacologic manipulation with 5-HTP (50-125 mg/kg I.P.) and DMT (10 mg/kg I.P.). Mortality, frequency and type of seizures did not differ significantly between drug and saline-treated animals. However, gerbils treated with 5-HTP showed a delay in the time of onset of seizures, whereas those treated with DMT seized earlier than the saline-treated controls. Experience with ischemia-induced seizures therefore supports previous studies in other epilepsy models which indicate an influence of serotonin on seizure threshold. (This work was supported by NIH NS 09287-07)

424 UNIT RESPONSES IN THE CAUDATE NUCLEUS DURING FOCAL PENICILLIN EPILEPTIC DISCHARGES. Charles M. Chuman*, E.J. Neafsey, Arthur A. Ward, Jr., Department of Neurological Surgery, University of Washington, Seattle, Washington 98195

Recent studies (e.g., Collins, et al. Arch. Neurol.,33:536, 1976) using the 2-deoxy-D-glucose method have demonstrated prominent activation of the basal ganglia during penicillin evoked epileptic discharge, confirming earlier observations using depth EEG recordings (e.g., Udvarhelyi and Walker, Arch. Neurol., 12:333,1965). The experiments reported here have extended these observations to the single unit level in an effort to understand more fully the processes involved in the spread and generalization of epileptic activity.

Experiments were performed on locally anesthetized, paralyzed cats. A 2 x 2 mm gelfoam pledget saturated with sodium penicillin (50,000 u/cc) was applied over cruciate sulcus. EEG was recorded at and remote from the focus with silver ball electrodes. Single unit activity was recorded in the ipsilateral caudate nucleus (A17,L5). Recording sites were verified histologically. Histograms of unit activity before ("background") and after interictal EEG spikes were accumulated on a CAT 400 computer. All 8 cats studied thus far showed similar responses.

Of the 108 caudate cells studied, 101 responded to cortical epileptic discharge. Five of these responded with bursts only during the clonic phase of seizures and were otherwise silent. The remaining 96 responded to cortical interictal EEG spiking as well, 78 with an initial high frequency burst of spikes and 18 with an initial decrease in firing rate. The burst in the caudate cells occurred at the onset of the cortical EEG spike. For 8 of the 78 bursting cells, the burst was followed by a long lasting (>500 msec) depression of firing rate. This figure (8/78) may be an underestimation because only 16 of the 78 bursting units had background firing rates high enough to permit detection of decreases, even with averaging. Of the 18 cells initially responding with a decrease in firing rate, 6 showed a later "rebound" increase in rate above their background activity.

In sum, the firing pattern of nearly all cells in the rostral portion of the head of the caudate nucleus is strongly affected by cortical epileptiform discharges. 82% fire a burst with at least 1/10 of these showing a later depression. 18% are initially decreased in firing rate with 1/3 of these showing a later "rebound" increase in firing. The dramatic effect of cortical epileptic discharge on the caudate nucleus suggests that this structure plays an important role in the subcortical spread and possible generalization of focal epileptic discharge.

425 EFFECT OF MATERNAL RESPERINE AND CHLORPROMAZINE ADMINISTRATION ON SEIZURE SUSCEPTIBILITY AND INTENSITY IN OFFSPRING RATS. John W. Dailey and Phillip C. Jobe, Department of Pharmacology, L.S.U. Medical Center, Shreveport, Shreveport, Louisiana, 71103.

Administration of reserpine or chlorpromazine to pregnant rats has been shown to impair the development of the restrained cold stress response in their offspring. This defect may be due to alterations in the development of noradrenergic neurons (Fed. Proc. 35: 585, 1976; Pharmacol. 18 (2): 331, 1976). Since noradrenergic systems have been shown to be important in the modulation of seizures (JPET 184: 1-10, 1973; Neuropharm. 13: 961-968, 1974) experiments were designed to determine if offspring from similarly treated dams have altered susceptibility to sound-induced seizures or if they have alterations in electrically-induced seizure patterns. Pregnant Sprague-Dawley rats received daily subcutaneous injections of either 7 mg/kg of chlorpromazine or 0.1 mg/kg of reserpine from day 8 of pregnancy until the pups were weaned at approximately 20 days postpartum. After they were more than 60 days of age, offspring from control and drug treated groups were exposed to a standardized sound stimulus. Offspring from reserpine treated dams had a seizure frequency significantly higher than control ($P<0.05$). Offspring from chlorpromazine treated dams were not different from control. Responses to supramaximal electroshock which was administered through corneal electrodes were not different between control and reserpine or chlorpromazine pretreated animals. There was also no difference among the groups in their response to electrically-induced spinal cord seizures. These data suggest that restrained cold stress is a more sensitive indicator of the reserpine and chlorpromazine induced neuronal deficits in these animals than is seizure susceptibility and intensity (Supported in part by National Foundation Grant #1-496).

426 EPILEPTIC CHICKENS AS A PHARMACOLOGICAL MODEL OF GRAND MAL EPILEPSY. H.L. Davis*, D.D. Johnson, and R.D. Crawford*. Dept. of Pharmacology, University of Saskatchewan, Saskatoon, Saskatchewan Canada S7N OWO.

Crawford reported a mutant causing epileptiform seizures in domestic fowl (J. Hered. 61: 185, 1970). Seizures can be evoked by intermittent photic stimulation (IPS). Acute dose-response studies with phenobarbital (PB), phenytoin (PH) and primidone (Pr) were conducted to determine the relationship between plasma levels and anticonvulsant effect in epileptic chickens. Complete protection against IPS-induced seizures was obtained with plasma PB levels > 14 µg/ml. Maximal anticonvulsant activity was obtained with plasma PH levels of 16 µg/ml, however complete protection could not be obtained at sub-toxic doses. Pr produced a significant reduction in seizure incidence for 24 h but is metabolized to PB. Concurrent administration of SKF-525A with Pr to prevent the generation of PB resulted in significant protection against IPS-induced seizures indicating that Pr itself possesses anticonvulsant activity. Phenylethylmalonamide (PEMA) levels of 49 µg/ml did not affect the incidence of seizures. The data revealed a high correlation between plasma PB, PH or Pr levels and anticonvulsant activity indicating that the epileptic fowl resemble human epileptics in terms of plasma anticonvulsant concentrations required for control of grand mal seizure activity. (Funded in part by an MRC post-doctoral Fellowship and MRC Grant #MA5893).

427 EFFECTS OF SEIZURE-PRODUCING STIMULATION ON PROTEIN PHOSPHORYLATION IN MEMBRANES FROM RAT CEREBRAL CORTEX. Y.H.Ehrlich, L. G. Davis* and E. G. Brunngraber. Mo. Instit. Psychiat., Univ. of Mo.-Columbia, Sch. Med., St. Louis, MO 63139.

Massive firing of neurons can be recorded in cerebral cortex following treatments that induce seizures, such as electroconvulsive shock or decapitation. Numerous studies have related this seizure activity to the increase in cerebral cAMP levels which are induced by these treatments. Cyclic AMP is known to exert its physiological functions by regulating phosphorylative activity. We have therefore investigated the phosphorylation of specific proteins in membrane preparations from rats in which cerebral cAMP levels were altered by seizure-producing stimuli. Osmotically shocked P_2 fractions were prepared from the cerebral cortex of rats sacrificed by head immersion in liquid nitrogen, and these served as controls. Corresponding fractions were prepared from cerebral cortex of rats whose head was immersed in liquid nitrogen after ECS or decapitation (experimental). Aliquots from the control and experimental groups were incubated with 3uM gamma-^{32}p-ATP for 10 sec. in the presence and absence of 5 uM cAMP. The specific protein substrates which incorporated labeled phosphate were identified by autoradiography of SDS-solubilized reaction products, electrophoretically separated in slab polyacrylamide gels. The results were quantitated by densitometric measurments of the autoradiograms. Specific differences which occurred in opposing directions were observed between the experimental and control groups. For example, phosphate incorporation into a protein band with approximate MW of 52K increased about two-fold after decapitation, while that of a band with MW = 47K showed a 50 % decrease. In fact, the ratio of phosphate content in these 2 proteins was inversed after treatment. It was 1.83±0.16 in the controls and 0.43±0.09 in the experimental group ($F = 72.74$; df= 1,8; $P < 0.001$). In addition, a marked stimulation by cAMP of the phosphorylation of two other bands (MW = 78K and 56K) was in evidence in membranes from seizured-cortex but not in controls. We have reported recently that endogenous phosphorylative activity towards the specific proteins mentioned above is characteristic for membranes of synaptic origin (Neurochem. Res., in press). It is possible, therefore, that the selective alterations in their phosphorylation as described here, are related to the changes in neuronal activity which occur during seizures. An inherent abnormality in protein phosphorylation systems may be investigated as one of the possible causes of epilepsy. (Supported in part by a grant from the Epilepsy Foundation of America).

428 ENDURING DOPAMINE DEPLETION AT THE SITE OF AMYGDALOID KINDLING STIMULATION. Jerome Engel Jr. and Nansie S. Sharpless* Depts. Neurol., Neurosci., & Psychiat., Albert Einstein College of Medicine, Bronx, NY 10461

Daily stimulations of the amygdala with brief currents that initially produce little or no behavioral responses gradually result in the development of generalized seizures. This kindled epileptogenicity is enduring since the typical seizure can still be evoked in kindled animals after many months without stimulation. The mechanisms accounting for these enduring alterations are unknown, although previous work in our laboratory and others has indicated a role for the catecholamine projection systems in the kindling phenomenon. In the present study, electrodes were implanted into the left amygdalae of 19 rats and 9 rats were kindled with daily amygdaloid stimulation until generalized seizures were evoked on three consecutive days, while 10 rats were handled daily but received no stimulations. One month after the last kindled seizure (kindled rats) or last handling trial (implanted control rats), the rats were killed, the brains removed, and noradrenaline (NA) and dopamine (DA) were measured radioenzymatically in both right and left amygdalae and hippocampi. NA and DA levels in the left (stimulated and/or implanted) and right structures were compared using Student's t-test for matched pair data. When compared to the right, nonstimulated side, there was a very significant decrease in both DA ($P<0.0005$) and NA ($P<0.005$) levels in the left, stimulated amygdala of the kindled rats. DA levels in the left amygdalae of the implanted control animals were not different from those in the right amygdalae. However, the NA levels were significantly lower ($P<0.05$) in the left amygdalae of the control animals. No left-right differences were found in the hippocampi of either group. When the mean percent difference between right and left amygdalae for kindled rats was compared with that for the implanted controls, there was a significant difference for DA ($P<0.05$) but not for NA. We conclude that amygdaloid kindling caused a consistent, specific, and persistent decrease in DA concentration in the left, stimulated amygdalae while the effect of amygdaloid kindling on NA concentration may not be greater than that produced by the electrode implantation alone. It is suggested that an enduring stimulation-induced decrease in DA mediated inhibition may be one mechanism underlying kindled excitability.
Supported by NIH Grants No. NS 09649 and NS 70209.

429 ALTERATION OF SINGLE UNIT RESPONSES TO VISUAL STIMULI IN THE RETICULAR FORMATION AND LATERAL GENICULATE INDUCED BY PENTYLENETETRAZOL. Carl L. Faingold and James Stittsworth*. Division of Pharmacology, Department of Medical Sciences, Southern Illinois University School of Medicine, Springfield, IL 62704.

Previous findings from this laboratory have demonstrated that pentylenetetrazol (PTZ) administration markedly alters the sensory responsiveness of units recorded in the mesencephalic reticular formation (MRF) (Faingold and Caspary, Neuropharm., 16:143, 1977). This study compared the effects of PTZ on visual responsiveness of units recorded in the MRF to the effects of this agent on the responsiveness of units simultaneously recorded in the lateral geniculate nucleus (LGN). Experiments were performed in acutely prepared, locally anesthetized, paralyzed cats. The data were examined using post-stimulus time histograms (PSTH's) with a bin width of 1 msec utilizing 50 presentations of light flashes at 1 per sec intervals. The results indicate that MRF units which did not respond to the visual stimuli became responsive after PTZ as shown by the emergence of the time-locked consistent peaks in the PSTH's. Units in the LGN were quite responsive to visual stimuli, and most of these units showed only minor modifications of the late components of the firing pattern after PTZ. These changes included the appearance of small multiple peaks at 100 to 200 msec after the stimulus. Some LGN units showed small increases in the height of the initial peak of the PSTH, while others showed no detectable change in firing pattern. Major modifications of MRF unit firing patterns were noted at subconvulsive doses of PTZ. However, changes observed in the responses of LGN units remained relatively minor even if PTZ was given to the point of seizure induction. The relative difference in the degree of PTZ-induced change in single unit responses to visual stimuli in MRF and LGN sites may explain previous findings which indicate that convulsant drugs enhance sensory evoked field potentials in non-primary sites to a much greater extent than in primary sensory sites (Faingold, Neuropharm., 16:73, 1977).

(Supported in part by funds from Southern Illinois University Foundation)

430 MATHEMATICAL PREDICTION OF HUMAN POST-TRAUMATIC EPILEPSY. Dennis M. Feeney and A. Earl Walker*. Depts. Psych., Physiol. and Neurosurg., Univ. New Mexico, Albuquerque, N.M. 87131.

The following equations estimate the probability of post-traumatic seizures given risk factors and for time between 1 wk and 5 yrs after the injury.

$P_n = P_0 (.925)^n$ where: /Equation 1/

n = number of months since the injury

P_n = probability a patient will have a seizure after time n

P_0 = estimate that a patient will have a seizure any time after brain injury, considering relevant risk factors. Let $P_0 = R_I$ and R_I is calculated using Equation 2.

.925= a constant estimating the probability that a patient will not have a seizure in any given month.

$R_{i+1} = R_i + \theta (1.2 - R_i)$ where /Equation 2/

initially; R_0=.01, lowest probability of post-traumatic seizures

θ = increment of R by a risk factor, the values are given below. Use no more than 4 in decreasing order of magnitude.

1.2 = a constant to adjust the final R or R_I to fit published data. So, with a limit of 4 factors, $.01 \leq R_I \leq .74$.

θ	Factor	θ	Factor
.05	unconsciousness/amnesia≥1 hr	.20	missile wound/dura tear
.10	persisting EEG abnormality	.05	linear skull fracture*
.20	hemiplegia, aphasia	.10	depressed skull fracture*
.20	hemorrhage (intracranial)	.25	central/parietal damage**
.15	fits in first wk	.15	temporal damage**
.10	prefrontal/occipital damage**	.10	infection (CNS)

*do not use with missile wounds unless dura intact.

**with multiple brain damage use the single highest θ.

Example: occipital wound, depressed fracture, 2 hrs coma (Equa.2)

R_1-depressed skull fracture θ=.10;R_1=.01+.10(1.2-.01)=0.129

R_2-occipital damage θ=.10;R_2=.129+.10(1.2-.129)=0.236

R_3-unconsciousness θ=.05;R_3=.236+.05(1.2-.236)=0.284

R_I=0.284 the probability of a fit any time after the injury. The probability of fits after 6 mos has declined and is calculated using Equation 1 with n = 6 and P_0 = 0.284.

$P_6 = 0.284 (.925)^6$

P_6 = 0.18 = the probability that the patient will have a seizure some time beyond 6 mos post-injury.

To determine the time to reach a given risk level, use Equation 1 and set P_n to desired risk and then solve for n.

These formulae, using a constant probability model, give results that fit published data and predict with 95% confidence the chance of post-traumatic epilepsy in single cases.

431 EFFECTS OF MONOAMINERGIC CHANGES ON POSTDECAPITATION CONVULSIONS IN RATS. P. F. Geiger, E. Greg Tubre*, P. C. Jobe. School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209, and Department of Pharmacology and Therapeutics, Louisiana State University School of Medicine, Shreveport, LA 71130.

The present study investigated the role of spinal cord (SC) monoamines in convulsions (C) induced by decapitation in rats. The magnitude of postdecapitation C was quantitated by use of two single-plane acceleration transducers (Grass Instruments). Reserpine (5 mg/kg i.p. 24 hours prior to decapitation) treated animals exhibited a convulsive magnitude which was 0.7% that of normal saline treated subjects. Also, reserpine decreased the duration of convulsive activity from 0.29±0.04 minutes (controls) to 0.04±0.01 minutes. These effects of reserpine were determined at 24 hours postadministration, a time when maximal depletion of norepinephrine (NE), dopamine (DA), and 5-hydroxytryptamine (5-HT) is also observed. Two drugs which have relatively specific effects on 5-hydroxytryptamine stores were also tested: parachlorophenylalanine (316 mg/kg i.p. 3 days prior to decapitation) a potent inhibitor of tryptophan hydroxylase; and 5,7-dihydroxytryptamine (10 µg of the base intracerebroventricularly 10 days prior to decapitation) a substance which produces a neurotoxic effect on 5-hydroxytryptaminergic neurons. Neither of these drugs produced an effect on either the magnitude or the duration of postdecapitation C. In contrast, 6-hydroxydopamine (200 µg of the base intracerebroventricularly) produced an effect on postdecapitation C which was qualitatively and quantitatively similar to that of reserpine. This effect was determined 14 days after 6-OH-DA administration, a time when SC NE levels are markedly decreased. These data indicate that noradrenergic neurons in the SC may play a role in the modulation of postdecapitation C.

432 FOCAL PENICILLIN EPILEPSY IN AN ISOLATED HEMISPHERE: EEG AND INTRACELLULAR STUDIES. J. J. Hablitz and D. V. Wray*. Neurophys. Dept., The Methodist Hospital, Houston, TX 77030.

The cortex of an entire cerebral hemisphere was surgically isolated in the cat to allow assessment of the role of intrinsic regulatory mechanisms in focal epileptic activity. Topical application of small amounts of penicillin to the cortex has been shown to result in a spatially, well-delimited focus producing recurring interictal spikes. Neurons at the center of such foci are strongly excited during the paroxysmal discharge while cells in the surrounding cortex and controlateral homotopic cortex are markedly inhibited during the interictal discharge. We now report rapid spread of epileptic activity in isolated cortex associated with decreased cellular inhibition.

Prior to surgical isolation of the hemisphere a small penicillin focus was created on the marginal gyrus and spread of activity assessed by closely spaced EEG electrodes. After removing the pledget and rinsing the cortex with saline the hemisphere was isolated by sectioning all forebrain commissures and dividing the white matter at the level of thalamus and caudate nucleus. Animals were then allowed to recover for 3-5 days. Subsequently, a focus was created on the suprasylvian gyrus and spatial spread studied. Intracellular recordings were then made at the focus or at distances removed from it.

EEG recordings indicated that interictal spike activity in the normal cortex was maximal in the focus and markedly reduced in amplitude at distances of 5-7 mm. Activity in the isolated hemisphere was similar in morphology to the normal cortex but was characterized by the presence of large amplitude spikes 10-15 mm away from the focus. These distant spikes appeared early in the development of the focus and are not attributable to diffusion of penicillin. Intracellular recordings at the center of the focus and at distances up to 10 mm showed typical large amplitude depolarizing shifts with superimposed high frequency spike activity and spike inactivation (paroxysmal depolarizing shifts, PDPs).

Hyperpolarizing shifts were noted to follow termination of PDPs in most cells; considerable variation in amplitude and duration was seen. In the small number of cells sampled to date (N=50) no uninjured neurons were encountered which displayed purely hyperpolarizing responses without prior activation.

These results indicate that interictal spikes in isolated cortex are associated with the same patterns of cell discharges seen in intact brain. The greater spread of interictal spikes suggests that this preparation may be useful for studying intrinsic control mechanisms.

433 EPILEPSY AFTER ALUMINA EXCISION IN THE ABSENCE OF "MIRROR FOCI" A. Basil Harris and Joan S. Lockard. Department of Neurological Surgery, University of Washington, Seattle, Washington, 98195.

The role of intracortical alumina in sustaining experimental epilepsy in monkey and the matter of secondary independent epileptic foci are examined in this study. Staged surgical excisions were performed on 15 monkeys clinically epileptic 3 to 5 years. Four to six months elapsed between each procedure to allow stable seizure frequency observations. The first operation was the removal of the alumina granuloma and immediate 1 mm. of surrounding cortex. The second procedure was removal of a 1 to 1½ cm. rim of cortical tissue which surrounded the first excision site. The final procedure was removal of a 1 to 2 cm. circle of homotopic cortex followed by profusion of the brain. Serial EEGs were performed and each animal was monitored on videotape or polygraph to prove the frequency or lack of seizures. Electrocorticography was performed to map areas of epileptic activity at each operative procedure. Histological studies were done on each excised piece of cortical tissue and, finally, on the profused brains utilizing Cajal's astrocyte stain, Nissle and histochemical stains for aluminum.

In the majority of animals (13 of 15), the first excision resulted in a greater than 50% decrease in seizure frequency, but in only two did the epilepsy disappear. The second excision stopped seizures in all but two animals, one of which had subdural scarring from the operative procedure. The second animal received a third resection which terminated epileptic activity. In no animal was aluminum detectable after the first excision. Cortical astrocytes were hypertrophied throughout the epileptic tissue up to the peripheral border of the second excision. Contralateral or homotopic areas of cortex did not show astrocytic hypertrophy. No independent "mirror foci" were seen by EEG or electrocorticography. Alumina generates the epileptic focus by scarring in the cortex, but it is not necessary to maintain seizures once they have become established. This series of long-term epileptic animals did not have independent homotopic epileptic foci. (Supported by NINCDS Grants NS-09037, NS-04053 and Contract NO1-NS-1-2282).

434 EFFECT OF TAURINE ON INTRACEREBRAL ELECTROSHOCK THRESHOLDS IN AUDIOGENIC AND NON-AUDIOGENIC RATS. Ryan Huxtable* and Hugh Laird. Depts. Pharmacol. and Pharmacol. & Toxicol., Cols. Pharm. and Med., Univ. of Az., Tucson, Az. 85721.

Audiogenic (AS) and non-audiogenic (NAS) rats were bilaterally implanted in the inferior colliculi (IC) with bipolar electrodes for determination of intracerebral electroshock thresholds (IET). IET is defined as the minimum current, in μA, required to elicit consistently a convulsion. Graded intensities of electrical current administered as 60Hz sine-wave pulses of 5 seconds duration were used to obtain stable IET values in each side of the IC in all subjects. Following IET determinations, groups of AS and NAS rats were injected with taurine (Tau) into either the lateral cerebroventricle (LCV) or directly into the IC. Lateral cerebroventricular injection of Tau (8μmoles) into AS rats elicited a biphasic effect on IET values. Initially (3 min) Tau elevated IET 44% but this response returned to baseline at 1 day only to be followed on day 2 by a long-lasting increase in IET. The IET values for 2,3,5, and 6 days were 34%, 61%, 64%, and 64% respectively. By day 7 the IET had returned to baseline. The LCV injection of Tau also reduced the severity of the convulsions displayed by the AS animals but only at the 3 min time period. In contrast to the AS subjects, LCV Tau administration did not alter either IET values or seizure severity in NAS animals. The injection of Tau (200nmoles) directly into the IC caused a slow developing but persistent elevation of IET in AS rats. The initial IET elevation was obtained 12 min after Tau at which time there was a 70% increase. The peak effect was noted (99%) 24 hrs after injection, and a significant elevation in threshold persisted for 20 days. Even though there was a marked and long-lasting elevation of IET in the AS animals, at no time was there a reduction in seizure severity. By comparison, injection of Tau into the IC of NAS rats did not cause a change in either IET nor seizure severity at any time after injection. These data show that Tau exerts long-lasting anticonvulsant effect only in AS rats, a species that is genetically susceptible to seizures. Furthermore, Tau administered LCV produced a different response to Tau given IC, indicating that the site of action was not the same. Supported by USPHS HL 19394 and 20087.

435 EFFECTS OF RO 4-1284 (RO) ON AUDIOGENIC (AG) SEIZURE (S) SUSCEPTIBILITY (SP) AND INTENSITY (I) IN RATS. P.C. Jobe, John W. Dailey and R. Don Brown. VA Hosp and Dept of Pharmacol, L.S.U. Sch Med, Shreveport, LA 71130.

This study determined effects of CNS biogenic amine depletion on AG S SP and I in 2 types of Sprague-Dawley derived rats: the progeny of genetically susceptible parents (PGS) and of nonsusceptible parents (PNS). S SP was determined by comparing the number of animals responding to sound stimulation [with a running episode only (RE) or with running episodes and convulsions (RC)] to the total number of animals tested. S I was assessed by determining an AG response score (ARS) for each animal (each higher ARS represents a more intense S). Three PGS subgroups were selected for experimentation on the basis of responses to sound stimulation: Subgroup 1 (SG1) showed no signs of AG S SP (ARS=0) in 3 pretreatment tests (at least 1 week apart); Subgroup 2 (SG2) animals had an RE (ARS=1) in 1 or 2 of the 3 test sessions and had an ARS=0 in at least 1 session; Subgroup 3 (SG3) animals had an ARS=1 in all 3 test sessions. PNS were selected for use in the same way as SG1 of PGS. The table shows S SP and I values prior to treatment (controls), 45 min after RO (a time when monoamine depletion is maximal), and 19 days after RO (18 days after depletion has completely dissipated). These results show that effects of RO on PNS are minimal. In contrast, the drug caused a increase in SP and I in all 3 PGS subgroups. In the PGS tested 19 days after RO, a relatively high degree of S activity was still present. These observations suggest that CNS monoaminergic neurons function as determinants of AG S SP in animals which also carry some other genetically determined SP factor. A deficit in monoaminergic transmission is insufficient to cause SP in animals not carrying this other factor.

TEST GROUP		CONTROLS (Before RO)			RO (45 Min. After)			AFTER RO (19 Days Later)		
		SP		ARS	SP		ARS	SP		ARS
		RE	RC		RE	RC		RE	RC	
PNS		0/23	0/23	0.0	3/23	1/23	0.2	1/22	1/22	0.1
PGS	SG1	0/17	0/17	0.0	1/17	10/17	5.4	4/17	3/17	0.7
	SG2	6/6	0/6	.33	0/6	6/6	9.0	1/6	3/6	1.1
	SG3	2/2	0/2	1.0	0/2	2/2	9.0	0/2	2/2	2.0

436 ANISOMYCIN DELAYS AMYGDALOID KINDLING IN RATS. Viliam Jonec*, Susan Holm*, David Masuoka* and Claude G. Wasterlain* (SPON: Richard N. Lolley). Epilepsy Research Laboratory, VA Hospital, Sepulveda, CA 91343, Brain Research Institute and Department of Neurology, UCLA, CA 90024.

Twenty-six adult Holtzman rats received one daily electrical stimulation to the basolateral amygdala (500 uA, 1 sec, 62.5 Hz, 5 days/week) through chronically implanted bipolar stainless steel electrodes. Experimental animals received an intraperitoneal injection of anisomycin (60 mg/kg) 30 min before each stimulation. Preliminary experiments showed that this dosage inhibited rat brain protein synthesis by over 75% during the first hour after injection and produced no apparent toxicity. Little inhibition was observed later than two hours post injection. Control animals were injected with solvent (0.9% NaCl). Handling and stimulation were similar in controls and experimentals. Clinical response and electroencephalogram (shape and duration of afterdischarge) were recorded with every stimulation. Kindling was continued until the animal responded to five consecutive stimulations with stage 5 seizures (Racine, R.J., EEG clin.Neurophysiol. 32:, 281, 1972). Twenty-four hours after the last stimulation, the animals were killed and dopamine, norepinephrine and serotonine concentrations were measured in both cerebral hemispheres, cerebellum and brain stem.

Anisomycin-treated rats reached each stage of kindling more slowly than saline-injected animals, and development of stage 5 seizures was delayed ($P < 0.001$). Crossover of treatments after kindling revealed no anticonvulsant action of anisomycin. One possible interpretation of these findings is that enzyme induction is necessary to the establishment of the kindling process.

437 CEREBELLAR MODULATION OF HIGH PRESSURE NERVOUS SYNDROME SEIZURES P. G. Kaufmann and J. C. Farmer, Jr.*, Duke University Medical Center, Durham, N.C., U.S.A.

The high pressure nervous syndrome (HPNS) in mammals proceeds from jerky movements of the limbs to tremors and myoclonic jerks and finally to generalized convulsions. Subjective reports by human divers have also included a vestibular involvement, manifested by dizziness, nausea, ocular and limb tremors, and standing unsteadiness. Electronystagmographic evidence indicated that these symptoms are not due to unilateral dysfunction of vestibular end organs (Farmer, et al., Undersea Biom. Res., 1974, 1, A-11). It was postulated that decreased cerebellar modulation of brainstem vestibular nuclei could result in the reported symptoms. In view of existing reports that the cerebellum may play a role in inhibition of epileptic activity (Cooper et al., J. Amer. Geriat. Soc. 1974, 24, 40-43), we further examined its role in the development of HPNS seizures.

Electroencephalographic activity of the frontal cortex, cerebellar vermis, vestibular nuclear complex, hippocampus, and reticular formation was recorded in awake rats during simulated dry chamber ($He-O_2$) dives to 136 atmospheres at 40 to 80 atm/hr. Effects of compression varied from severe tremor and myoclonic jerks to tonic-clonic convulsive seizures and status epilepticus. The predominant seizure pattern observed was a spike-and-slow-wave pattern of 4-5/sec. Cerebellar ablation two weeks prior to the dive significantly lowered seizure threshold and increased the number of convulsions, but had no effect on the electrographic pattern evident during seizures. (Supported by ONR Contract N00014-75-C-0553).

438 EFFECT OF CONVULSANT DRUGS ON PHOTIC AFTERDISCHARGE. Gary A. King* (SPON: W.M. Burnham). University of Toronto, Department of Pharmacology, Toronto, Canada.

Photic Afterdischarge (PhAD) consists of a series of high amplitude, low frequency (6-8Hz), slow waves that appear in the visual cortex following normal light-flash evoked potentials. It usually lasts for 1-2 seconds.

Recent work on PhAD indicates a number of similarities to petit mal epileptic seizures: a) it often has a spike-and-wave pattern; b) it is suppressed by the arousal state; c) it is suppressed by anti-petit mal drugs but unaffected by anti-grand mal drugs; d) during its occurrence some behavioural responses are inhibited.

Work by previous experimenters has shown that PhAD is enhanced by metrazol. The present series of experiments, a dose-response investigation of the effects of picrotoxin, bicuculine and strychnine, in rat, indicates that it is also enhanced by drugs which interfere with GABA transmission in the CNS.

439 LOCAL CHANGES IN PO_2 AND PREDICTION OF METABOLIC RATE DURING INTERICTAL ACTIVITY IN EXPERIMENTAL PRIMARY AND SECONDARY EPILEPTOGENIC FOCI. Norman R. Kreisman, Thomas J. Sick* and Duane F. Bruley*. Departments of Physiology and Chemical Engineering, Tulane University, New Orleans, La. 70112.

Focal epileptogenic activity was produced in one hemisphere of the bullfrog telencephalon by microinjection of 10-100U of Na penicillin G. The electrocorticogram (ECoG) was monitored from each hemisphere with bipolar Ag/AgCl electrodes. Measurements of local PO_2 in one or both hemispheres were made polarographically with glass insulated, platinum-iridium microelectrodes with a tip diameter less than 1μ and an exposed tip length of 15-20μ.

The most frequently observed responses consisted of slow transient decreases in PO_2 which followed the penicillin-induced ECoG spikes and gradually recovered to control values with an exponential decay. The duration of these transient decreases in PO_2 ranged from 10-60 sec and the magnitudes were as large as 35mm Hg. As the frequency of ECoG spikes increased with further development of the penicillin focus, the PO_2 decreases summated, driving the PO_2 to lower values which often approached 0 mm Hg. Slowing of the ECoG spikes always resulted in a return of PO_2 levels to a value higher than control suggesting that penicillin or the interictal paroxysms may produce hyperemia. When the ECoG spikes were followed by afterdischarges the resultant PO_2 decreases recovered to control values more readily than before and were followed by a large overshoot. In contrast, small slow waves, often resembling afterdischarge with no preceding interictal spike, were followed by transient increases in PO_2. Simultaneous recordings from two polarographic microelectrodes revealed that the ECoG related PO_2 transients occurred intitally in the 1° focus and several minutes later in the 2° focus. Most often these transients occurred for varying periods of time in one hemisphere and then the other. This alternation appears analogous to the "flip flop phenomenon" first described by Morrell (Epilepsia 1:538, 1960).

Distributed parameter, non-linear partial differential equations representing oxygen convection and diffusion were used to calculate local metabolic rates based upon the PO_2 transients. The equations were solved using a pseudo dynamic technique on a hybrid computer by the parallel solution method. These mathematical models predict metabolic rate changes which are inversely proportional to the changes in PO_2 and directly proportional to superimposed simulated increases in local blood flow. Direct measurements of local blood flow are necessary to test the predictions of these models.

This work was supported by NIH grant NS-12419.

440 INTRACEREBRAL ELECTROSHOCK THRESHOLDS OF AUDITORY NUCLEI IN AUDIOGENIC AND NON-AUDIOGENIC RATS. Hugh E. Laird and Ryan J. Huxtable* (SPON: Lincoln Chin). Depts. Pharmacol. and Toxicol., and Pharmacol., Colleges Pharm. and Med., Univ. Ariz., Tucson, Ariz. 85721.

Intracerebral Electroshock Thresholds (IET) of the medial geniculate bodies (MG), inferior colliculi (IC) and ventral cochlear nuclei (VCN) were determined in groups of audiogenic (AS) and non-audiogenic (NAS) rats. The IET is defined as the minimum electrical current, in μA, required to cause a behavioral convulsion. Each subject was bilaterally implanted in one pair of auditory nuclei with bipolar stimulating electrodes. IET values were determined on each side by administration of graded intensities of electrical current as 60Hz sine-wave pulses of 5 seconds duration. Subjects were tested daily on alternate sides until stable thresholds were obtained. Stimulation of the auditory nuclei at the IET current elicits in both the AS and NAS rats behavior identical to that observed in the AS animal during sound-induced seizures. However, there is a marked difference in the convulsive pattern displayed by two strains of rat. The AS animal consistently shows a maximal (tonic extension), whereas the NAS animal displays only a minimal type of convulsion. Another difference between the AS and NAS subjects was observed in the IETs of the auditory nuclei. IET values in the MG, IC, and VCN of AS rats were significantly lower than the comparable thresholds in NAS subjects, as shown in the table.

INTRACEREBRAL ELECTROSHOCK THRESHOLD

LEFT	IC	MG	VCN
AS	43 ± 7	73 ± 14	48 ± 9
NAS	184 ± 39	525 ± 24	>1000
RIGHT			
AS	49 ± 7	112 ± 48	43 ± 8
NAS	193 ± 46	400 ± 39	>1000

These results may be summarized as follows: (1) Electrical stimulation of auditory nuclei in both AS and NAS rats elicited an audiogenic seizure response; (2) The AS animal had a more severe convulsive seizure; (3) IET values in all auditory nuclei are markedly lower in the AS rat. Supported by USPHS grants HL 19394 and HL 20087.

441 PATTERNS OF DEPTH SPIKING RELATED TO PSYCHOLOGICAL ASSESSMENT IN PATIENTS WITH EPILEPSY. Jeffrey P. Lieb, Rebecca Rausch*, and Paul H. Crandall*. (SPON: J. Rohrbaugh). Reed Neurological Research Center, UCLA, Los Angeles, CA 90024.

The statistical properties of depth spiking in the interictal EEG were assessed in 12 patients with intractable temporal lobe epilepsy in whom depth electrodes had been implanted for diagnostic purposes. Prior to the implantation surgery each patient was administered standard psychological tests. Following electrode implantation in medial temporal lobe depth sites, 1 to 4 EEG recording sessions, varying in length from 29 to 300 minutes, were analyzed with a computerized automatic spike recognition system in each patient. The mean rate of spike occurrence was measured bilaterally in the amygdala, pes hippocampi, and hippocampal gyrus. The total amount of spike activity in each patient (TA) was measured by computing the root mean squared mean spike rates across all sites bilaterally. The degree of lateralization of spike activity (LAT) in each patient was measured by computing the ratio of the root mean square of spike activity in the more active temporal lobe to TA. TA and LAT were correlated with the pre-implant psychological measurements. TA negatively correlated with intelligence (i.e. Wechsler Full Scale, Verbal and Performance I.Q.s). LAT, while not significantly related to the intelligence scores, correlated positively with a general memory score (i.e. the Wechsler Memory Quotient) as well as a rating of psychosocial independence. Age, sex, length of seizure history, or drug serum levels at the time of psychological testing did not correlate with the behavioral measurements.

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442 EFFECTS OF 1,4-BENZODIAZEPINES ON ANTIDROMIC INVASION INTO THE GIANT NEURON OF APLYSIA CALIFORNICA. M.F. Murphy* and N.R. Kreisman (SPON: A.W. Epstein). Depts. of Pharmacology and Physiology, Tulane University, New Orleans, LA 70112.

Repetitive neuronal discharge along synaptic pathways is an acknowledged mechanism for the spread of ictal activity and the establishment of secondary epileptogenic foci in mammalian CNS. The projection of this aberrant activity may occur in an orthodromic fashion or by antidromic invasion of normal soma through ectopic spike generation in nerve terminals of axons within primary foci (Gutnick & Prince, Science 176:424, 1972). The giant neuron of the invertebrate Aplysia (R2) can serve as a model for a presynaptic terminal due to its dependence on sodium and calcium for spike generation and its unique axon arborization at the spike trigger zone near the soma. Retrograde invasion into R2 by axon stimulation was assessed in the presence of two 1,4-benzodiazepine derivatives: diazepam, a psychotropic which aborts ictal spread and prevents the establishment of secondary epileptogenic tissue, and R05/4864 (7-chloro-5-(p-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepine-2-one), a derivative devoid of significant pharmacologic effects in all mammalian test systems. Two KCl filled microelectrodes were inserted into the soma of R2, and the second connective, containing the axon of R2, was secured over a bipolar stainless steel electrode. One microelectrode was used to rigidly control the transmembrane potential of the cell just prior to axon stimulation while the second microelectrode recorded membrane potential during antidromic invasion. Stimulation of the right connective with a 20V, 0.5 msec pulse every 3 min yielded reproducible axon and soma spikes. Bath application of diazepam (10^{-4}M) produced an increase in latencies to both axon and soma spikes, an increase in soma spike width, and a marked decrease in soma spike height and afterhyperpolarization which was maximal at 1/2 hr post application. R05/4864 (10^{-4}M) for 1 hr produced either no effect or marginal changes opposite in direction to that seen with diazepam. The effects of diazepam and R05/4864 were reversible only with prolonged washing.

The effects of diazepam suggest a possible mechanism of membrane stabilization which could produce conduction blocks in regions of low safety factor such as points of axon bifurcation and dendritic arborization. Conduction block mechanisms could abort seizure spread and prevent the establishment of secondary epileptogenic foci independently of effects on neuronal activity in primary foci. (Supported by NIH Fellowship 4-F02-GM52664, the Southern Medical Association Research Project, and NIH grant NS-12419).

443 SYNCHRONOUS DISCHARGES IN NEOCORTEX ALTER THE EXCITABILITY OF INTRACORTICAL AXON TERMINALS. Jeffrey L. Noebels and David A. Prince. Dept. Neurol., Stanford Univ. Sch. Med., Stanford CA 94305.

During epileptogenesis produced by focal application of penicillin or strychnine to cat cortex, action potentials generated in the intracortical axons of thalamocortical relay (TCR) cells antidromically invade their parent cell bodies (Gutnick and Prince 1972, Schwartzkroin et al. 1975, Scobey and Gabor 1975). We have found that, in 89% of the TCR cell population of n. ventralis posterolateralis, the brief (20 msec) bursts of antidromic spikes following each isolated interictal discharge are greatly prolonged in duration (.1-1.5sec) during the interictal-ictal transition period. At this stage, axonal spike firing accounts for a majority of TCR cell activity, and is directly associated with the development of focal seizures. In order to determine whether the excitability change initiating repetitive firing in axons is dependent upon direct exposure of the intracortical axon terminals to convulsant drugs, or whether it might be a result of epileptiform activity in cortex per se, we studied the activity of identified TCR cells in nucleus ventralis posterolateralis of the cat during seizure activity initiated by brief trains of electrical pulses applied to postcruciate cortex. During the early stages of the cortical afterdischarge, bursts of regular (3-4msec) interval, constant amplitude spikes were recorded in TCR cell bodies. Burst spikes were proven to be of antidromic origin by demonstrating that they did not collide with cortically-evoked antidromic spikes. Antidromic bursts occurred only during afterdischarges, and could not be produced by subthreshold cortical stimulation. Intracellular recordings showed that antidromic bursts were often present in TCR cells immediately before the prolonged cellular depolarizations associated with each cortical discharge, indicating that prior orthodromic activity in the neuron was not necessary to trigger axon bursts. Within 10 sec of the onset of continuous cortical seizure activity, axonal spike generation ceased abruptly without any decrease in firing frequency. Since no synaptic contacts onto the intracortical segment of TCR axons are present, the results show that axon terminal excitability is modulated by endogenous events coincident with neocortical discharges. These and other data show that non-synaptic excitation and repetitive firing of intracortical axons occur in normal cortex during intense neuronal discharges, and may be presumed to be a general mechanism for increasing synchronization and excessive excitatory synaptic drive in cortical epileptogenesis. (Supported by NIH grant NS06477 from NINCDS.)

444 SELECTIVE ATTENUATION OF TRANSMITTER-INDUCED CHLORIDE CONDUCTANCE BY PENTYLENETETRAZOL (PTZ). Terry C. Pellmar and Wilkie A. Wilson. Epilepsy Center, Veterans Administration Hospital and Department of Physiology and Pharmacology, Duke University Medical Center, Durham, NC 27705

In the neurons of Aplysia californica, the synaptic actions of low concentrations (2 to 10 mM) of PTZ were studied. Iontophoretic responses were elicited by acetylcholine, dopamine, serotonin and GABA. Chloride-dependent, fast inhibitory responses to the neurotransmitters were reduced 50 to 60% by 2 mM PTZ. At the same concentration, PTZ caused an attenuation of the sodium-dependent, fast excitatory response by approximately 25% and of the potassium-dependent, slow inhibitory response by approximately 10%. The extent of attenuation of the responses did not vary with the neurotransmitter used to elicit them. Higher concentrations of PTZ (10 mM) further reduced all of the responses. At the concentrations of PTZ used, membrane actions were minimal. Two millimolar PTZ had no effect on the current-voltage (I-V) relationship of the cells while ten millimolar PTZ induced a small degree of non-linearity into the I-V curve.

These results indicate that the most potent action of PTZ is an attenuation of chloride conductance elicited by neurotransmitters in the cells of Aplysia. Since these effects are very similar to those previously described for penicillin (Pellmar and Wilson, Brain Res. 1977) a decrease in synaptic chloride conductance is postulated as a general mechanism of convulsant activity.

445 DICHOTOMOUS RESPONSE TO PENICILLIN-INDUCED INSTABILITY IN THE ALPHA-CHLORALOSE ANESTHETIZED CAT. S.R. Quint*, J.D. Charlton* and R.N. Johnson. Depts. Biomed. Engr. and Neurol., Sch. Med. Univ. of Virginia, Charlottesville, Virginia 22901

Active regulatory mechanisms have been implicated in the control and arrest of seizure activity, with subcortical sources of regulation activated by corticofugal influence. We have previously compared the effects of stimulation of subcortical structures and of common anticonvulsant drugs on excitability of the thalamocortical motor system. In this study we have investigated the influence of an active penicillin focus on the excitability of the thalamocortical motor system of the α-chloralose anesthetized cat.

Stimuli were presented in pairs to ventrolateral thalamus, varying both the amplitude of the second stimulus and the stimulus pair period. Resultant evoked responses from the computer generated stimuli were quantified and stored on-line. Multidimensional augmentation curves were then used to characterize the excitability of the system in its control state and after penicillin application.

Following the application of penicillin to cortex, homotopic to the recording site, one of two developments was observed, with equal incidence. In one group, an initial, unequivocal suppression of the response profile was accompanied by a relatively quiescent electrocorticogram (E.Co.G). Despite persistence of the epileptogenic focus, the response magnitude consistently returned to control levels, followed by considerable cortical spike activity and occasional seizurelike episodes of complete E.Co.G. disruption. Such intense epileptiform activity was followed by a return to the suppressed profile, an elevated threshold, or both. This modification of excitability suggests the presence of an active regulatory mechanism. The alternate development to the attenuated response was represented by a consistently magnified response profile, accompanied by moderate cortical spiking. However, intense epileptiform activity was never observed. The magnified response to the focus appears contingent upon a fundamental difference in the pre-penicillin response profile. These control profiles were obtained at a deeper level of anesthesia where augmentation showed less complex behavior. We assume that the mechanisms responsible for the complex modification of augmentation are not operative when this peculiar chloralose state is manifest. There is considerable evidence suggesting that cortical depression from α-chloralose may eliminate feedback to subcortical structures with an increase in convulsant threshold, while subcortical structures are facilitated, with resultant motor hyperexcitability. Such a selective elimination of corticofugal influence would provide a plausible explanation for the dichotomous response to penicillin-induced instability.

446 CONVULSANT ACTION OF MONOFLUORACETIC ACID: CORTICAL DISINHIBITION. W. Raabe and L. Zieve*. Dept. of Neurology, St. Paul-Ramsey Hospital, St. Paul, Minn. 55101, and Dept. of Research, VA Hospital, Minneapolis, Minn. 55417.

Monofluoracetate (MFA), which blocks the oxidation of citrate in the tricarboxylic acid cycle, is a well-known convulsant. However, the detailed mechanism of convulsant action is not clear. Because MFA increases cerebral ammonia concentrations just prior to convulsions it was suggested that the effects of ammonia, abolition of postsynaptic inhibition, initiate the convulsion.

The effects of intravenously administered MFA and ammonium acetate (AA) on cortical postsynaptic inhibition and cerebral ammonia concentrations were studied. Pentobarbital anesthetized and artificially respirated cats were used. Extracellular recordings were obtained from pyramidal tract cells. Cortical postsynaptic inhibition was measured as the efficacy of recurrent postsynaptic inhibition of pyramidal tract cells to suppress antidromic action potentials of pyramidal tract cells. As soon as the efficacy of inhibition was abolished the widely exposed cortical hemispheres were frozen with liquid nitrogen. Cerebral ammonia concentrations were determined with the Conway microdiffusion method.

As the first sign of neuronal toxicity MFA (1 mg/kg bodyweight) abolished the efficacy of cortical post-synaptic inhibition; at this time an electrocorticogram still showed no seizure patterns. MFA induced disinhibition occurred at cerebral ammonia concentrations (345 ± 275 S.D. nmol/gm wet weight, N=6) which were only slightly higher than in sham-operated animals (284 ± 191 S.D. nmol/gm wet weight, N=12). AA (1.4 - 3.3 mmol/kg bodyweight) abolished the efficacy of cortical inhibition at ammonia concentrations about three times higher (861 ± 156 S.D. nmol/mg wet weight, N=5) than in sham-operated animals.

MFA abolishes the efficacy of cortical post-synaptic inhibition like ammonia does. The effect of MFA on inhibition occurs independent of MFA induced increases of cerebral ammonia concentrations because MFA-disinhibition occurs at almost unchanged cerebral ammonia concentrations whereas AA-disinhibition requires about three times increased cerebral ammonia concentrations. Cortical disinhibition may account for the convulsant action of MFA.

447 *NEUROPHYSIOLOGICAL AND BEHAVIORAL CHANGES PRODUCED BY CEREBELLAR STIMULATION.* Lee T. Robertson, Mary Artero*, and Don Rushmer. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, Oregon 97209.

Electrical stimulation of the cerebellar cortex is being used for therapeutic control of selected cases of epilepsy and cerebral palsy. In monkeys (Macaca fascicularis) we have used similar electrode systems and placement in an attempt to quantify the physiological parameters and long-term stability of stimulation and to identify behavioral changes. The monkeys were trained to precisely touch three buttons in a left-to-right sequence. The analyses of the forelimb movement was by EMG, single-frame motion picture, and computer printouts of timing characteristics for each trial. The monkeys were then implanted with modified Avery electrodes over the intermediate cerebellar cortex and bonescrew electrodes for EEG were symmetrically placed over the cranium. Evoked responses were averaged (N=100-250) by a PDP-12 computer. The lowest threshold was for bilateral cerebellar stimulation, which produced a large amplitude evoked potential over the sensory-motor cortex. The shape of the potential consisted of two biphasic positive-negative waves; at higher current levels the amplitudes of the first two waves increased and a third positive wave also appeared. The shape of the response was consistent at various frequencies but an increased amplitude occurred at higher current levels or longer pulse durations. Contralateral stimulation required a slightly higher current threshold and ipsilateral stimulation produced no response. Cerebellar stimulation consistently affected the wave form of sensory evoked potentials. For example, one minute of bilateral stimulation surpressed the late component (23-40 msec) of an evoked response produced by electrical stimulation of the tail. The effect lasted up to five minutes. One week of continuous stimulation (1 min on/off) did not affect the shape or amplitude of any of the evoked responses. Bilateral cerebellar stimulation at slightly above threshold had no effect on the precisely timed forelimb movement. However, at slightly higher current levels a significant increase in the latency of the movement occurred during and immediately after stimulation. Contralateral stimulation produced only a small effect and ipsilateral stimulation resulted in no change. High-speed films of the movement indicated that stimulation produced a decrease in the velocity but did not disrupt the stopping, starting, or precision of limb placement. Possibly a beneficial effect of cerebellar stimulation in cerebral palsy patients is a slowing of phasic movements.

448 A RODENT MODEL OF CENTRENCEPHALIC EPILEPSY. Paul F. Robinson* and Shirley A. Gilmore. Dept. Anat., Univ. Arkansas Med. Sci., Little Rock, AR 72201.

A subpopulation of Charles River CD^{R} albino rats has been found to display behavior possessing certain characteristics of human petit mal epilepsy and of generalized penicillin epilepsy in the cat. These characteristics include cessation of spontaneous movement, staring, bilateral twitching of the vibrissae, nose and mouth, and augmentation by drowsiness. The ECoG tracings of these animals display trains of polyspike plus spike and wave discharges with average frequencies of 6-10 complexes per second and amplitudes of 300-700 μv. The ECoG findings differ from reports by McQueen and Woodbury (Epilepsia 16: 295, 1975) and Fariello (Epilepsia 17: 217, 1976) of the inability of the rat brain to produce spike and wave forms.

Such discharges appear spontaneously in approximately 20% of the subpopulation. They may be induced in the remainder of the subpopulation by parenteral injections of pentylenetetrazol (10 mg/kg body weight) and penicillin G (100,000 u/kg body weight/day for 1 to 5 days). Those discharges which are spontaneous or penicillin-induced are self-sustained in that they persist throughout the six-month duration of the study.

449 BRAIN EXTRACTS AND AUDIOGENIC SEIZURES: ENHANCEMENT OF SEIZURE RATE BY EXTRACTS FROM DBA/2J MICE AT PEAK SEIZURE SUSCEPTIBILITY. Robert A. Schreiber, Dept. Biochemistry, Univ. of Tennessee Center for the Health Sciences, Memphis, TN 38163.

Mice susceptible to sound-induced seizures (audiogenic seizures, AGS) have been considered an experimental model for the study of sensory epilepsy.

One major question is whether susceptible mice are synthesizing some unusual substance which makes them hyperreactive, or whether they lack some necessary substance which affords sufficient protection under normal circumstances. An experimental approach to this question is to prepare a brain extract from selected AGS susceptible or non-susceptible donor mice, inject it into selected recipients, and test the recipients for AGS. (It is assumed that any active fraction contains a substance not present in inactive fractions.)

The experiment reported here is the first attempt to alter susceptibility to AGS in a strain of mice intrinsically susceptible to AGS at particular ages. (Other published efforts to confer heightened sensitivity to acoustic stimulation have been performed on C57BL/6J mice subjected to audiogenic priming. Of these, only one demonstrated significant differences on an initial test for AGS in recipient mice[1].)

Previous data do not exclude the possibility that the substance of interest is a peptide. Brains of 21-day-old DBA/2J mice were removed and lyophilized. The freeze-dried brains were powdered then extracted with cold 1.0 M acetic acid for 2 hr, and centrifuged for 1 hr at 5° at 6000 rpm. The supernatant was lyophilized, taken up in .05 ml H_2O per mg of brain powder, and centrifuged as before. The supernatant was passed through a PM10 Amicon filter, again lyophilized, and resuspended in 0.20 ml H_2O per brain equivalent. Recipient 21 day old DBA/2J mice were injected i.p. with one brain equivalent, and tested for AGS at either 2 hr, 1 day, or 2 days, or 5 days after injection. No mouse was used more than once. The incidence of lethal seizures was significantly ($p<.05$) raised from a control level of 36% to an experimental level of 81% in mice tested 1 day later. There were no significant differences in any level of seizure observed between experimental and control (untreated) mice tested at 2 hr, 2 days, or 5 days. This experiment supports the hypothesis that some additional substance is present in the brain during a period of CNS hyperreactivity.

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[1]Schreiber, R. A. & N. N. Santos. Pharm. Bioch. Behav., in press.

450 SOME NEUROCHEMICAL CORRELATES IN SEIZURE PRONE GERBILS. R. N. Seaman*, S. L. Seaman and A. Y. Sun*. Sinclair Comparative Medicine Research Farm, University of Missouri, Columbia, MO 65201.

The Mongolian gerbil (Meriones unguiculatus) has previously been reported to exhibit a spontaneous seizure response similar to that found in the human epileptic. Over 50% of the gerbils in our colony displays an epileptiform seizure characterized by (1) ear and vibrissae twitch, (2) eye blinking, (3) body tremor, (4) crouching, (5) myoclonic body jerks, (6) clonic-tonic seizures, (7) rapid running and finally a (8) quiescent period. The seizure was initiated either by handling or by exposing the animal to a foreign environment. The latency, duration and severity of the epileptiform response was variable. This behavioral condition appears to be inherited as an autosomal, dominant condition with incomplete penetrance. Three groups of female gerbils (10-14 months old) were injected intraventricularly with 5 μCi [^{14}C]-GABA and 5 μCi of [^{3}H]-NE. The animals were then sacrificed by decapitation five minutes after the radioisotope injection. The control, non-seizing animals displayed the greatest in vivo brain uptake of NE and GABA. The biogenic amine uptake was significantly lower in those animals injected prior to the onset of the seizure. A significant difference in NE-uptake was also observed between the pre- and post-seizure injected groups. In another experiment, male gerbils (10-15 months old) were decapitated during seizure and compared with non-seizure control animals. Seizing animals exhibited an elevated in vitro synaptosomal uptake of Ca^{2+}. There was no difference in synaptosomal (Na^{+} + K^{+})-ATPase or AChE activity. From these studies it appears that some membrane systems are altered during the seizure response. This animal model may prove useful in testing the neurochemical as well as physiological conditions resulting from epileptic seizure.

451 REDUCED SEIZURE SUSCEPTIBILITY TO MMH INTOXICATION FOLLOWING DENTATE AND VENTROBASAL THALAMIC LESIONS IN CATS. M. N. Shouse*, M. B. Sterman and J. M. Siegel. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024 and Neuropsychol. Res. and Neurophysiol. Res., VAH, Sepulveda, CA 91343.(SPON: Joel F. Lubar)

The present experiment attempted to clarify conflicting evidence on the relationship of 12-15 Hz sleep spindles to seizure activation. Seizure thresholds were calculated in minutes post-injection following IP administration of the convulsant drug monomethylhydrazine (MMH) to cats with lesions intended to enhance the occurrence of spontaneous 12-15 Hz sleep spindles recorded from sensorimotor cortex. Twelve cats with bilateral cortical and subcortical recording electrodes were divided into three groups receiving electrolytic lesions in the dentate nucleus (Group I), the ventrobasal (VB) thalamus (Group II), or in one of various "control" regions (Group III). Lesion sites in Group III animals avoided primary afferent pathways to VB thalamus, destruction of which has been found to enhance sleep spindle activity (Elliot and Sterman, Anatomical Record, 1975, 181: 352) and included cerebellar white matter, pyramidal tract, and ventral pontine tegmentum. Prior to the MMH trials, three baseline EEG's were obtained during pre- and post-lesion conditions. Following the MMH trial, lesions were verified histologically. Results of the MMH trial revealed that animals with dentate and VB thalamic lesions showed elevated seizure thresholds relative to those with control lesions. EEG analyses indicated that animals with reduced seizure susceptibility showed prolonged periods of slow wave sleep relative to their own pre-lesion baselines and to the pre- and post-lesion baselines of control animals. These findings are compatible with the view that sleep spindles do not facilitate seizure activation in epileptogenic neuronal populations (Wagner et al., Electroenceph. clin. Neurophysiol., 1975, 39: 499-506) and may, in fact, exert a protective influence, as suggested by our findings in both epileptics and patients with dorsal column injury.

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452 UNIT ACTIVITY AND LOCAL CHANGES IN PO_2 DURING FOCAL EPILEPSY IN THE FROG. Thomas J.Sick* and Norman R.Kreisman. (SPON: J.P. Ellison). Department of Physiology, Tulane University School of Medicine, New Orleans, LA 70112.

Unit activity and local PO_2 were recorded from the primordium hippocampus of bullfrogs by glass coated platinum-iridium microelectrodes with a tip diameter of <1μ and an exposed tip length of 15-20μ. Focal epileptogenic activity was initiated by microinjection of 10-100U of Na penicillin-G and the electrocorticogram was monitored from bipolar Ag/AgCl electrodes placed on the surface of each hemisphere.

Units were classified according to the occurrence of firing in relation to ECoG spikes. Of 34 units recorded from 14 bullfrogs, 18 were excited, 12 were inhibited, 2 showed excitation followed by inhibition and 2 were unaffected during or immediately following the ECoG discharge. The four unit response classes correspond well with those reported by Wilder and Morrell (Neurology 17:1193, 1967). Occurrence of unit firing was also compared to ECoG related PO_2 changes. The most common response was a transient (10-60 sec) decrease in local PO_2 following the ECoG spike. This was accompanied by a short (500-1000 msec) high frequency unit discharge, the duration of which often coincided with the duration of the falling phase of the PO_2 transient. Units which were inhibited following the ECoG discharge were correlated with PO_2 decreases only when an excitatory response from another unit was recorded at the PO_2 microelectrode. Units unaffected during the ECoG discharge were rare and were never accompanied by a change in local PO_2.

Association of local PO_2 transients with excitatory unit responses suggests that these transients reflect increased oxygen consumption by those units and/or by neighboring glial cells. However, since local blood flow was not measured, a transient decrease in local blood flow associated with the excitatory unit response cannot be ruled out.

This work was supported by NIH grant NS-12419.

453 INCREASED SENSITIVITY TO MAXIMAL ELECTROSHOCK SEIZURES FOLLOWING SELECTIVE DESTRUCTION OF NORADRENERGIC NEURONS WITH 6-HYDROXYDOPAMINE (6-OHDA). R. L. Simonton* and R. A. Browning. Southern Illinois University, School of Medicine, Carbondale, IL 62901.

Despite a vast amount of pharmacological evidence showing seizure inhibitory effects of the central catecholamines, the relative contributions of norepinephrine (NE) and dopamine (DA) remain somewhat equivocal. In order to evaluate the relative importance of NE and DA in this regard, seizure susceptibility was examined in rats after selective destruction of NE or DA neurons with 6-OHDA. Pretreatment with benztropine mesylate (30 mg/kg, i.p.) and intraventricular 6-OHDA (100 + 50 μg) resulted in a 55-60% depletion of forebrain NE without altering DA or serotonin. Animals subjected to maximal electroshock stimulation (MES) 30 days following treatment with the benztropine + 6-OHDA combination exhibited a decrease in the latency for hindlimb extension ($P < .02$), an increase in the duration of hindlimb extension ($P < .05$), and a prolongation of recovery ($P < .01$). Moreover, the ability of acetazolamide (12 mg/kg, i.v.) and phenobarbital (8 mg/kg, i.p.) to abolish hindlimb extension in the MES test was markedly reduced in rats treated with benztropine + 6-OHDA. In contrast, rats treated with protriptyline HCl (20 mg/kg, i.p.) and intraventricular 6-OHDA (200 μg) failed to differ significantly from vehicle-injected rats in their response to MES 30 days post injection. The anticonvulsant effects of acetazolamide and phenobarbital were likewise unchanged in the rats receiving protriptyline + 6-OHDA. Monoamine analyses in animals treated with protriptyline + 6-OHDA revealed a 50-60% reduction in forebrain DA with no deficit in NE. These findings show that a selective reduction in brain NE can increase seizure susceptibility and antagonize the action of anticonvulsants, while a comparable reduction in DA does not have this effect. Although these data do not preclude the possiblity that DA functions as a modulator of MES-induced seizures, it indicates that NE plays a greater role in this regard.

454 EXPERIMENTAL EPILEPSY AND CEREBELLAR STIMULATION IN THE MONKEY. Henry V. Soper, Jeffrey P. Lieb, Thomas L. Babb, George M. Strain* and Paul H. Crandall. Brain Research Institute, UCLA, Los Angeles, CA. 90024.

An alumina cream model of temporal lobe epilepsy was developed in the monkey to try to determine the effect of cerebellar stimulation on such epilepsy. Five monkeys had fresh alumina cream (aluminum hydroxide) stereotaxically placed in the anterior and posterior hippocampus in successive surgeries with 8½ months between the surgeries. None developed chronic epilepsy after the first placement. Three more received one-stage bilateral placements. All monkeys received recording electrodes and #32 and #33 received 6.8mm^2 platinum stimulating electrode pairs on the paravermal cortex.

In three weeks the animals became anorexic, and within the next two weeks they began having psychomotor seizures. Five animals started having relatively mild seizures almost continuously (epilepticus partialis continua), appeared almost comatose, and required force-feeding; one died from an unrelated cause; and two continued to have seizures until they were sacrificed (113 and 432 days after surgery).

Of the animals which went into status, three died, and two (#80 and #33) were deteriorating with no evidence they would recover. Cerebellar stimulation electrodes were implanted in #80, and both monkeys were stimulated at 2.4 uC/phase at 10 hz for 10 min on and 10 min off. Over the first four days the most dramatic change in #80 was the reduction in the spread of seizure activity from the hippocampus to the cortex. The general condition of the animal improved and it began having clear interictal intervals. Unfortunately, it choked on its food and died shortly after this improvement. The other monkey (#33) also began improving after four days. After 2½ weeks the behavior was normal during interictal intervals, although it was still having seizures. Immediately after cerebellar stimulation was terminated, the state of the animal remained the same, but over the next two months it deteriorated. Again, after four days of stimulation there was some improvement and after 2½ weeks interictal behavior was normal. Following the end of stimulation this time the behavior remained relatively stable for the next 11 months and subsequent stimulation did not alter it.

These preliminary results suggest that bilateral, multifocal, alumina cream implantations in the hippocampus can induce a chronic model of temporal lobe epilepsy. In addition, although cerebellar stimulation does seem to have an effect on this epilepsy, it appears to be most effective in the severely epileptic monkey, takes about four days to show any effect, and has its primary effect on improving the general interictal condition of the animal. Supported by NIH Contract NS 4-2331.

455 PREVENTION OF SEIZURE-INDUCED INCREASE IN BRAIN WATER IN THE DEVELOPING RAT BY STEROID DRUG THERAPY. Norma J. Starr and David Holtzman*. Dept. Neurology, Stanford U. School of Medicine, Stanford CA 94305

Previous studies in our laboratory have shown an increase in brain water and electrolytes, as well as a decrease in both brain and body weight in rat pups exposed to repetitive seizures between 11-15 days after birth. Furthermore, a frequent pathologic finding after prolonged seizures in young children is cerebral edema. If indeed the production of cerebral edema is involved in the encephalopathy resulting from repetitive seizures, then the use of drugs which reduce brain water, (e.g. steroids) may be useful in the management of young children who seize.

In order to study the effects of steroid drug therapy on animals subjected to seizures early in life, litters of rat pups were divided into weight-matched halves, with one-half receiving daily injections of either corticosterone (25 or 50 µg/g) or dexamethasone (1 or 5 ng/g) between 1-21 or 10-21 days of age. On days 11-15, 'seizure' litters received 2 electroshocks per day (150 V., 1 sec, AC) to study the effects of repeated seizures on brain weight and water content and body weight. Control litters, with animals receiving the same dose had the electrodes attached, but with no current passed.

Our results indicate that chronic treatment with a steroid drug can prevent the increase in brain water and the decrease in brain and body weights observed after seizures alone. Differences between the two drugs were seen in terms of the relative effectiveness of different treatment regimes. After corticosterone treatment (1-21 days) both the 25 and 50 µg doses could attenuate the increase in brain water and electrolytes, with no loss of brain or body weight. After treatment from 10-21 days, only the 50 µg dose was effective. Dexamethasone treatment, at both doses tested, and under both treatment schedules resulted in the prevention of the seizure-induced increase in brain water and changes in brain weight.

Thus it appears that chronic treatment with optimal doses of a steroid drug may be effective in the prevention of post-seizure encephalopathy in the developing brain. (Supported by (NIH Research Grants, NS 12151 and ES 01197 to D.H. and IT 32NS-02012-02 to N.S.)

456 EFFECTS OF CENTRAL CORTICAL EEG FEEDBACK TRAINING ON EEG CHARACTERISTICS AND SEIZURE INCIDENCE IN POORLY CONTROLLED EPILEPTICS. M. B. Sterman and L. R. Macdonald. V. A. Hospital, Sepulveda, CA 91343 and University of California, Los Angeles, CA 90024.

This study examined the effects of central cortical EEG feedback training on seizure incidence in poorly controlled epileptics. After baseline recordings patients were trained in the laboratory and then initiated on a double crossover, ABA design using portable equipment at home, with bimonthly laboratory test sessions. EEG data were recorded polygraphically and on magnetic tape. Performance at home was monitored by a strip chart recorder within the portable unit. Training was based upon the simultaneous detection of two central cortical (C_3-T_3) EEG frequency bands (6-9 Hz and either 12-15 or 18-23 Hz), with reward provided for the occurrence of one in the absence of the other. The ABA design used consisted of successive 3 month periods of training, with reward contingencies reversed after each period and without the subject's knowledge. EEG response evaluations (power spectral analysis) and seizure incidence records were compared before, during and after the ABA design.

Results to date indicate significant redistribution of EEG power spectral densities in direct relation to training contingencies, with reversed contingencies producing reversal in power spectral distribution. Epileptics were found to be consistently deficient in power above 8 Hz, and particularly in the 12-15 Hz band during stage 2 sleep. Six of the eight patients reported significant reductions in seizures initiated during or in strict relationship to reward for 12-15 Hz or 18-23 Hz in the absence of 6-9 Hz central cortical activity. EEG changes associated with seizure reduction reflected a normalization of waking and sleeping EEG patterns.

These data are interpreted as indicating that significant and specific changes in EEG characteristics, and therefore in the neural substrates of the cortical EEG, can be achieved in epileptics with operant conditioning procedures. The reinforcement of normal thalamocortical conduction patterns with certain training contingencies is felt to account for both the reduction in EEG abnormalities and the apparent elevation of seizure thresholds observed.

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457 THE EFFECTS OF PHENYTOIN ON SENSORY EVOKED RESPONSES IN THE CAT. James D. Stittsworth, Jr.,* Robert Teece* and Carl L. Faingold. (SPON: J.Donald Easton). Southern Illinois University School of Medicine, Springfield, IL 62708

The effects of phenytoin (PHT) administration on auditory and visual evoked responses were studied in unanesthetized, paralyzed cats. Responses evoked by visual or auditory stimuli were recorded from primary sensory structures (cortex, lateral geniculate, inferior colliculus) and reticular formation. Phenytoin was infused intravenously either in commercial diluent (consisting of 10% ethanol and 40% propylene glycol) or in saline solution at rates of 1-2 mg/kg/min. Arterial blood samples were drawn and analyzed for PHT serum levels, using gas-liquid chromatographic methods (Kupferberg, Clin. Clim. Acta.29:283, 1970). Complete and long-lasting abolition of all auditory evoked responses was seen with a total dose of 20-25 mg/kg of PHT in commercial diluent. Doses in a range of 22-56 mg/kg of PHT in saline were required to induce the same degree of reduction in auditory evoked responses. Although the doses required to cause the reduction of responses differed with the two solutions, the serum PHT levels were found to be consistently in the range of 30-40 µg/ml in both cases. At these serum levels of PHT primary visual evoked responses were not consistently altered. Although the responses evoked in reticular formation by auditory stimuli were abolished, the responses to visual stimuli in the same site were unaffected. Thus, PHT appears to have a preferential effect on the responses to auditory stimuli. The commercial diluent appears to enhance the effectiveness of PHT on auditory evoked responses. In addition, at the higher dose rate of PHT in the commercial vehicle EEG changes were noted that would be consistent with the induction of sleep. Since drowsiness is induced by PHT in patients only when it is administered intravenously, the diluent may play a role in the production of this symptom. Previous reports have shown that auditory evoked responses enhanced by the administration of the epileptiform "anesthetic chloralose are also abolished by PHT administration (Herman and Bignall, Electroenceph, clin. Neurophysiol. 23:351, 1967). Responses to sensory stimuli are also enhanced in a variety of other seizure models (Faingold, Neuropharm. 16:73, 1977), and the reduction of the auditory responses by PHT may play a role in the ability of PHT to reduce seizure susceptibility.

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458 EFFECTS OF CEREBELLAR STIMULATION ON SEIZURE THRESHOLDS. George M. Strain*, William G. Van Meter* and William H. Brockman* (SPON: J. P. Lieb). Biomedical Engr. Prog. and Dept. Vet. Anat., Pharm. and Physiol., Iowa State Univ., Ames, IA 50011.

Cerebellar stimulation (CBL) and standard anticonvulsant drugs were compared for their abilities to elevate seizure thresholds. Generalized EEG seizures were elicited in conscious, acute New Zealand albino rabbit preparations by 10 or 15 mg/kg intravenous (IV) injections of pentylenetetrazol (PTZ) or by electrical stimulation of the frontal cerebral cortex at 50 Hz, 1 msec monophasic pulse duration and 4.0-7.6 volts for 2 seconds (ELEC).

After establishing control seizures, one of three anticonvulsant treatments was applied: IV injection of (1) phenobarbital (PHE) 25 mg/kg or (2) diphenylhydantoin (DPH) 30 mg/kg, or (3) transhemispheral electrical stimulation of the simplex and ansiform lobes of the cerebellum. Seizures were repeatedly induced with regular increments in PTZ dose or ELEC voltage until a seizure was evoked that approximated the control in severity and duration.

Cerebellar stimulation parameters of 10 Hz, 1.5-2.0 msec biphasic pulse duration, and 3-4 volts were found to be effective in attenuating PTZ-induced EEG seizure activity. Deviation from these parameters markedly decreased the effectiveness of the stimuli.

Analysis used six blocks of data: elevations of ELEC seizure thresholds by PHE, DPH or CBL, and elevations of PTZ seizure thresholds by PHE, DPH or CBL. ELEC seizure thresholds were found not to be elevated by CBL, and PTZ seizure thresholds were not elevated by DPH. In each of the remaining four cases the seizure threshold elevations were significant at a level of $p < 0.025$ or greater.

Elevations of seizure threshold (percent change in dose or voltage) were compared to evaluate the efficacy of CBL in comparison with PHE and DPH as antagonists of generalized seizures. At a significance level of 0.05, the elevation of PTZ seizure thresholds by PHE was greater than that of CBL and greater than the elevation of ELEC seizure thresholds by DPH. No differences existed at the 0.01 level of significance.

The failure of CBL to elevate ELEC seizure thresholds was attributed to a possible need for chronic cerebellar stimulation and/or differences in the seizure-inducing mechanisms of PTZ and ELEC.

It is concluded that the inhibition of generalized seizure activity on an acute basis by CBL does not differ significantly from that seen with DPH or PHE.

459 EFFECTS OF ELECTRICAL STIMULUS INTENSITY ON HIPPOCAMPAL POST-ICTAL PHENOMENA. H.S. Swartzwelder*, C.M. Eccles* and R.S. Dyer. (SPON: L.D. Fechter). Dept. Environmental Health Sciences, Johns Hopkins Univ., Balto., Md. 21205.

Electrical Stimulation of the hippocampus may produce afterdischarges (AD's) which can be recorded in the absence of obvious behavioral manifestations. Little attention has been given to the electrical changes which occur immediately following an AD, or to the influence of stimulus intensity upon these changes. Casual observation has revealed that the latter stages of the well known post AD EEG flattening (depression) are often characterized by a brief period of bilateral synchronous spiking, which we call the rebound AD. The AD, depression and rebound are often characterized by the appearance of behavioral episodes similar to wet dog shakes known to accompany such phenomena as morphine withdrawal (Psychopharm., 1976, 46, 191) and generalized motor convulsions (Psychopharm., 1975, 44, 33). The present study was designed to determine the influence of stimulus intensity upon the properties of hippocampal AD and post-AD phenomena. Sixteen Long-Evans male rats had bipolar nichrome wires implanted into the dorsal hippocampi for stimulation and recording, and skull screws implanted for grounding. Two weeks were allowed for recovery, whereupon thresholds for producing AD's were determined by the method described by Racine (EEG, 32, 1972). The animals were then matched according to threshold levels and divided into two groups, one receiving daily stimulation at 115% of threshold, and the other receiving daily stimulation at 400% of threshold. Results indicated that the post-AD depressions associated with AD's in the high intensity group lasted significantly longer than did those associated with AD's in the low intensity group. High stimulus intensity was inversely related to AD duration. Wet dog shakes occurred most frequently during the rebound AD, and their frequency was not related to stimulus intensity, AD duration, depression duration or rebound duration. When present, these wet dog shakes tended to occur immediately following or during the latter third of the AD and rebound AD. This research was supported in part by NIH grants HL 054053 and EHS 00454.

460 DRUG EVALUATION OF EXPERIMENTAL PSYCHOMOTOR STATUS EPILEPTICUS: A NEW ANIMAL MODEL. Katherine Taber*, S.F. Zornetzer and B.J. Wilder. Dept. Neuroscience, College of Med. U. of Fla., Gainesville, Fl., 32610.

Recently a new animal model of psychomotor status epilepticus, produced by a variant of the kindling paradigm, was reported (EEG Clin. Neurophysiol., in press). Self-sustained seizures (SSS) were induced by the repetitive application of electrical stimulation via depth electrodes chronically implanted into the dorsal hippocampus of male Swiss ICR mice. The stimulus parameters used were a 1.0 sec pulse train at 60 Hz, using 1 ms square-wave biphasic pulses at an intensity of 400 ua (constant current). The interstimulus interval was 60 sec.

Application of 100 to 150 stimulations to subfield CA3 of hippocampus results in the induction of non-lethal SSS. These seizures continued unabated for several hours after termination of electrical stimulation. Non-lethal SSS is characterized electrophysiologically by a slow spiking of 2-3 Hz and a dome or spike and dome wave form. Behaviorally these mice exhibit frequent automatisms or stereotyped behavior. These characteristics parallel the behavioral and electrophysiological observations of human psychomotor status epilepticus.

To test further the validity of this proposed animal model for human psychomotor status, drug challenges with antiepileptic drugs were performed. A double blind format was employed for all drug trials. Drugs were administered IP 20 min. after the onset of SSS and observations were continued until the cessation of SSS. The effect of diphenylhydantoin (DPH) (20mg/kg), phenobarbital (25mg/kg) and trimethidione (150 mg/kg), were evaluated on the behavioral and electrophysiological manifestations of non-lethal SSS. Results support the hypothesis that non-lethal SSS is a useful model for human psychomotor status epilepticus in so far as the pharmacological response of SSS in the mouse closely parallels the human clinical data. The implications of these data for future experimentation will be discussed.

461 EFFECTS OF PHENOBARBITAL ON SEIZURE ACTIVITY IN THE GERBIL. Kathy S. Watanabe*, Richard J. Schain and Beva G. Bailey*. Depts. Pediat. and Neurol./NPI, Sch. Med., UCLA, Los Angeles, CA 90024.

Phenobarbital is used extensively in the treatment of febrile seizures and epilepsy. Its popularity stems from its reported lack of adverse side effects on major organ systems. However, little is known about its influence on the central nervous system. Of particular concern is the developing brain, whose vulnerability to external events is well documented. Previous work in this laboratory has demonstrated a brain growth retardation in immature rats subjected to chronic phenobarbital administration. The present study was concerned with the effect of chronic phenobarbital treatment on seizure behavior in a strain of Mongolian gerbils (Meriones unguiculatus) that is predisposed to spontaneous seizures. Subcutaneous injections of 60 mg/kg phenobarbital were given daily to the experimental animals from 1 to 5 months of age. Control animals received equivalent doses of vehicle. At the termination of treatment (5 mo.), animals were tested regularly for seizure susceptibility until one year of age. Phenobarbital-treated animals exhibited an intensification of seizure activity both during and after the period of drug treatment. All animals receiving phenobarbital had one or more spontaneous seizures during the treatment period; 67% of the controls seized. During this time phenobarbital-treated animals had an average of 10.5 seizures with a mean seizure degree of +3.2 (scale of 1-5), whereas the controls had an average of 3.5 seizures with a mean seizure degree of +2.4. During the entire 7 month post-treatment period, the phenobarbital-treated animals displayed a mean seizure degree of +3.4 as opposed to +2.3 for the controls; drug treatment gerbils exhibited more frequent unstimulated seizures (66%) than did controls (37%). The latency to seize was shorter for the phenobarbital-treated animals (142 sec.) than for controls (233 sec.), and experimental animals had seizures of greater duration (231 sec.) than did controls (107 sec.). (All comparisons are significant at the 0.001 level.) These studies reveal that chronic phenobarbital administration enhances seizure activity in the gerbil. This enhancement persists long after termination of phenobarbital treatment. The explanation for this paradoxical effect may lie in the effects of phenobarbital upon the development of brain inhibitory mechanisms.

462 EFFECTS OF ELICITED SEIZURES ON LEARNING IN THE PAPIO PAPIO BABOON. Susan B. Weinberger* and Eva K. Killam* (SPON: A. Gabor). Departments of Pharmacology, University of California, Davis, CA 95616 and Boston University, Boston, MA 02118.

The contribution of seizures to the performance deficits that have been reported in human epileptics has been difficult to assess directly. This question has therefore been investigated experimentally by evaluating the effects of elicited seizures on learning in the epilepsy-prone baboon, Papio papio. Using three prepubertal animal subjects, learning performance was measured with a repeated acquisition task that required the animals to learn and perform a different sequence, or chain, of lever presses on a three-lever behavior panel each day. For each animal, the length of the chain started at two elements each session and increased stepwise as mastery of a chain was demonstrated.

When seizures were elicited twice weekly in each animal over a period of three weeks, mean performance levels (as assessed by efficiency, total errors, and time to criterion for each chain length) did not differ from preceding control values. However, learning performance was found to be significantly better at 18 hours than at 42 hours after each individual seizure, suggesting some type of cyclic phenomenon. When seizures were elicited at 24-hour intervals for 7 days, this cyclic phenomenon could not be evaluated, but the performance data demonstrated no overall or cumulative effect of these more frequent seizures on learning. Additionally, no changes in the baboons' spontaneous behaviors were observed over the course of this study.

Since limited numbers of seizures did not produce sustained impairment of learning performance, it was concluded that sources other than the sequellae of seizures themselves should be considered as the cause of performance deficits in epileptics.

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463 EFFECT OF TRANSVERSE CORTICAL LESIONS ON AFTERDISCHARGE THRESHOLD, DURATION AND SPREAD. Howard J. Williams*, John H. Ferguson, M.D. and Gene H. Barnett*. (Spon: P. Bart Vrtunski). Division of Neurology, Case Western Reserve University School of Medicine and Cleveland Veterans Administration Hospital, Cleveland, Ohio 44106

A series of experiments were carried out to determine the effects of transverse (subpial) cortical lesions (TCL) of the cat suprasylvian gyrus (SS) on electrically induced Afterdischarge Threshold(ADT), Afterdischarge Duration (ADD), and Afterdischarge Spread (ADS).

Seventeen cerveau isole animals were studied. Lesions were performed 11 to 60 days earlier under sterile conditions. Platinum balled electrodes were placed over the SS bilaterally such that each gyrus was divided into two halves (four quadrants). Peak current ADT (50 Hz 5 sec train of biphasic pulses, 2 msec duration, 2 msec separation) for each quadrant was measured. Steps of 0.1 ma were used until ADT was obtained.

Results indicate that: 1. In the 6 control cats (no lesions) mean ADT was .57 ± .03 ma compared to a mean of .55 ± .03 ma in the 11 experimentals (1-8 transverse lesions). 2. ADD at threshold in control cats was 4.09 ± .52 sec compared to 11.93 ± 2.88 sec in experimentals. TCL did not seem to prevent ADS from stimulated lesioned areas, but did limit spread into lesioned areas from stimulation of non lesioned SS quadrants.

Contrary to data from chronically implanted and stimulated animals (Ferguson et al Neurosci. Abst. 2:245, 1976) the present experiments suggest that TCL does not significantly effect ADT. Furthermore, the fact that they increase ADD suggests an enhancement of epileptogenicity.

464 EPILEPTIFORM DISCHARGE INDUCED BY FERROUS AND FERRIC CATIONS. L. J. Willmore , G. W. Sypert , and J. B. Munson. VA Hospital Dept. of Neurology, Div. of Neurosurgery, and Dept. of Neuroscience, University of Florida, College of Medicine, Gainesville, Fl. 32610.

Application of epileptogenic agents such as penicillin, ouabain, and cobalt to pial surface of rat isocortex have been utilized to assess neural mechanism associated with the initiation of epileptiform discharge. However, the neuropathologic mechanisms associated with the development of human focal epileptic lesions may not be reproduced in models of epilepsy requiring such chemical application to brain. Implantation of metallic iron into monkey motor cortex induces activation-sensitive focal epileptiform discharge. This observation, combined with the known convulsant effect of subarachnoid injection of erythrocyte contents suggests that the presence of the metallic compounds of whole blood within cortical tissue may be important in epileptogenesis.

In the present study a solution containing 100 mM $FeCl_2$ (Fe^{++}) or $FeCl_3$ (Fe^{+++}) was iontophoresed on the pial surface of rat sensorimotor isocortex with a 5 μA current for 5 min from a pool of ionic solution contained within a plexiglass footplate having a 500 μM orifice. Electrocorticograms (ECoG) were continously recorded via bipolar metallic electrodes in contact with the pial surface ipsilateral to the site of iontophoresis. Multiunit extracellular recordings were obtained from various depths within the isocortex with a glass micropipette placed through the central perforation of the iontophoretic well.

After variable latency, high amplitude epileptiform discharge was observed in the ECoG. During iontophoresis of both ionic species extracellular microelectrode recording within isocortex revealed progressive unit synchronization at the time of epileptogenesis, with superficial layers of isocortex affected prior to the deeper lamina. Fe^{++}-induced epileptogenesis was associated with more prominent unitary burst activity than with Fe^{+++}. Furthermore, there appeared to be a greater disinhibition with Fe^{++} than Fe^{+++}. Depth of cation penetration was identified by specific histochemical staining for iron compounds.

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465 BURST GENERATION AND CALCIUM MEDIATED SPIKES IN HIPPOCAMPAL CA3 NEURONS. Robert K.S. Wong* and David A. Prince. Dept. Neurol., Stanford Univ. Sch. Med., Stanford CA 94305.

The finding that hippocampal CA3 neurons act as pacemakers for epileptogenic discharge in the penicillin-treated slice preparation led us to study the behavior of guinea pig CA3 neurons in the normal slice. In contrast to CA1 cells, a large proportion of CA3 neurons exhibit spontaneous bursting activity. Bursts consisted of 2-11 spikes riding on a slow depolarization of up to 27mV. In bursts of more than 3 spikes, 2 phases of activity could be recognized. The early phase consisted of 2-4 rhythmic spikes whose interspike interval, spike height, and duration showed little variability from burst to burst in a given cell. Events in the late phase of the burst at the peak of the slow depolarization consisted of single or repetitive slow spikes with multiple humps or peaks. These spikes had longer durations (4-5msec), smaller amplitudes (up to 30mV), and longer, more variable, interspike intervals than the first few spikes of the burst. Short duration (2msec) intracellular depolarizing current pulses could evoke the whole train of spikes. Brief intracellular hyperpolarizing pulses of sufficient intensity to block a single spike, applied at various intervals after the first spike of a burst, would abort the subsequent spikes in the train. This and other evidence suggest that the burst-generating mechanism is an intrinsic property of CA3 pyramidal cells. When slices were treated with TTX (10^{-6}g/cc), intracellular depolarizing current pulses evoked slow spikes (4-5msec duration) with multiple humps and peaks. These TTX-resistant spikes appeared at levels of membrane depolarization comparable to those which evoked spikes in the late phase of the burst. Increasing the stimulating current decreased the latency of these responses and elicited rhythmic repetitive spike activities of increasing frequency. The slow high threshold spikes were reversibly blocked by locally applied Mn^{++}. In control experiments we found that single but not repetitive TTX-resistant spikes could be evoked in CA1 neurons; no such potentials have been seen in granule cells to date.

These data suggest that Ca^{++}-mediated spikes in CA3 pyramidal cells participate in normal bursting behavior. There is a striking similarity between the bursts studied and those recorded in CA3 and CA1 neurons during penicillin epileptogenesis in the slice. There is also a parallelism between the capacity to generate Ca^{++} spikes and the degree of involvement in penicillin epileptogenesis among the 3 major cell groups in the hippocampus. We would speculate that generation of Ca^{++} spikes may contribute to development of the depolarization shift and spike bursts in hippocampal "epileptic" neurons. (Supported by NIH research grant NS06477 from NINCDS.)

466 STATISTICAL ANALYSIS OF SINGLE UNIT DISCHARGES IN GENERALIZED PENICILLIN EPILEPSY. D. V. Wray* and J. J. Hablitz (SPON: R. P. Borda). Neurophys. Dpt., The Methodist Hospital, Houston,TX 77030.

Generalized paroxysmal discharges in cortical and subcortical structures can be induced in cats by parenteral administration of penicillin. Spontaneous bursts of paroxysmal activity occur against a background of otherwise normal activity. Microelectrode analysis of the paroxysms has revealed the presence of altered neuronal activity; we now report statistical studies which suggest altered single unit-EEG relationships during time periods not encompassed by paroxysms.

Extracellular single unit recordings were obtained from neocortex and cerebellum of awake cats with simultaneous recording of cerebellar and cortical EEG activity. Paroxysmal events in cerebellum were associated with increased unit activity in 39% of all cells (N=59) analyzed while excitatory/inhibitory sequences were seen in 30.5%. Seven percent showed a non-specific slowing during cerebellar paroxysms, while the remainder were not affected. In cortical recordings, approximately 80% (N=283) demonstrated altered firing rates during paroxysms. Ongoing activity was interrupted and replaced by bursts of action potentials coincident with the spike portion of the paroxysm followed by cessation of activity during the wave. For statistical analysis artifact-free segments of EEG activity (80 sec) were randomly sampled by a digital computer and the amplitudes obtained sorted into discrete voltage categories. The same segment of EEG activity was then systematically sampled at times related to the occurrence of each single unit discharge. Comparison of the unit related histogram to the random histogram is then made.

Using quantitative comparison of statistical data (mean, median, mode) generated by these procedures, it was found that during penicillin epilepsy cortical EEG activity was significantly related to the time of occurrence of cortical unit discharges, a relation which did not obtain in the normal non-penicillin state. Similarly cerebellar EEG was correlated with cerebellar unit activity. Analysis was also made of the relation of cerebellar unit activity to cortical unit discharge; significant correlations were obtained. These effects were seen prior to time of occurrence of spike discharges rather than after as might be expected if simple propagations were involved. No consistent relation between cortical EEG and cerebellar unit activity was seen.

These results demonstrate the usefulness of a statistical approach to analysis of single unit data, and suggest that not only is there a significant relationship between paroxysmal events and neuronal activity in generalized penicillin epilepsy but an overall trend to closer synchronization of EEG and unit discharge.

EXTRAOCULAR MOVEMENTS

467 UNITS IN THE SUPERIOR COLLICULUS AND UNDERLYING TEGMENTAL STRUCTURES RESPONDING TO PASSIVE EYE MOVEMENT. V. C. Abrahams and G. Anstee*. Department of Physiology, Queen's University, Kingston, Ontario, Canada. K7L 3N6.

In previous experiments (Rose and Abrahams, Brain Research, 97, 95, 1975) a quantitative study was made of passive, nasally directed eye movements that led to unit activity in the superior colliculus and which was presumed to derive from receptors in extraocular muscle. At the time a population of units was examined which was only excited by large eye movements, usually greater than 20°. A second population of units were also found in the superior colliculus which was excited by small eye movements. The response of this type of unit have now been examined in detail as has the response of units to vertical as well as horizontal movements.

An opaque contact lens was cemented to the cornea of one eye of a chloralose anaesthetized cat and this lens in turn was cemented to a device which could be used to produce vertical or horizontal movements in either direction at controlled velocity and from any initial position. The presence of large displacement threshold units was confirmed and they were found to respond to both vertical and horizontal movements. The majority of units in the superior colliculus have now been found to respond to small eye movements. Movement threshold ranged from 2 to 8° and was initiated by an applied corneal force of 1 to 15 g. Response was independent of initial eye position, and movement in either direction (upward or downward, right to left, or left to right) normally caused the same unit to discharge. The units so activated lie mainly in the intermediate and deep layers of the superior colliculus with some in the underlying tegmentum. Cells of origin of the tectospinal tract lie in this area and can be antidromically activated by stimulation in the contralateral upper cervical cord. Sixty per cent of cells so identified respond to passive eye movement.

One group of units were found located in the region of the mesencephalic nucleus of V and the tegmentum lateral to this nucleus which respond to passive eye movement. Attention was first drawn to these units because of their short latencies and great sensitivity. Horizontal movements of the eye of less than one degree and a load on the cornea of less than one gram is sufficient to activate these units. The response is not of retinal origin as it was present after the optic nerve had been severed. Responses were not elicited by corneal touch with Von Frey hairs with thresholds up to one gram. It may be that this eye movement induced activity may arise in jaw muscle spindles, a possibility strengthened by the fact that these same units are extremely sensitive to vibration.

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468 EXCITATORY AND INHIBITORY EFFECTS OF DORSAL NECK AND EXTRAOCULAR MUSCLE AFFERENTS IN FRONTAL EYE FIELD REGIONS IN THE CAT. H. Barbas, B. Dubrovsky, D. Williams* and M. McCormack. Neurophysiol. Lab., Allan Mem. Inst., McGill Univ., Montreal.

We have shown that dorsal neck and extraocular muscle afferents project and converge onto neurons in regions corresponding with the frontal eye field (FEF) of the cat (Dubrovsky and Barbas Exp. Neurol. 1977, In Press). We now report that these muscle afferents also have inhibitory effects on FEF neurons, as was shown by testing these stimuli against a background of neural activity induced through iontophoretic release of glutamate. A sample of 115 neurons was recorded either in response to peripheral nerve stimulation or to iontophoretic release of glutamate. These included 83 units which responded to stimulation of one or more dorsal neck and/or extraocular muscle nerves, 22 which were inhibited, and 20 which were not affected by these muscle afferents. Out of 70 units tested, 56 responded to both extraocular and dorsal neck muscle afferents. This degree of convergence (80%) onto FEF neurons was higher than that observed with stimulation of the nerves of different dorsal neck muscles (22%), and two or more extraocular muscle nerves (59%). Although neurons in the FEF generally responded to more than one of the muscle nerves tested, subsets of this sample appeared to be selectively affected by pairs of extraocular and/or dorsal neck muscle afferents. Thus, whereas 9 units were excited with stimulation of the superior rectus on one side, they were inhibited by the homologous contralateral muscle nerve. Excitatory and inhibitory responses of 4 FEF neurons were also observed with stimulation of two groups of dorsal neck muscles, which involved, respectively, the contr. biventer cervicis/complexus, and the contr. rectus capitis dorsalis major/obliquus capitis caudalis muscles. Another 4 FEF units were inhibited by the contr. biventer cervicis/complexus of the neck, and also by either the contr. lateral rectus, or the contr. superior rectus muscles of the eye. Also, 5 units were inhibited by one extraocular or dorsal neck muscle nerve, but were otherwise unresponsive to any of these afferents. The latencies of excitation by one input and those of inhibition by another overlapped in 36% of the cases and were 8-35 ms, while in 20% of the cases inhibition occurred later. Inhibition of units which were otherwise unresponsive to any dorsal neck or extraocular muscle afferents occurred either as early as 10-20 ms, or as late as 45-180 ms. The majority (70%) of the units were situated within the upper and lower lip of the lateral cruciate gyrus. The converging input of afferents from muscles that move the eyes and the head, and their specific excitatory and inhibitory interactions onto these FEF regions suggest a role of these afferents in coordinated eye-head movement.

469 EFFECT OF REARING IN STROBOSCOPIC ILLUMINATION ON FIXATION AND SMOOTH EYE MOVEMENTS OF CATS. Janet L. Conway*, George T. Timberlake*, and Alexander A. Skavenski* (SPON: Frank H. Duffy). Dept. of Psychology, Northeastern University, Boston, MA 02115.

Rearing in a stroboscopically illuminated environment disrupts the velocity analyzing capacity of cat cortical and collicular neurons by denying experience with continuous retinal image movement. We examined whether the types of eye movements known or suspected to be driven by retinal slip velocity would also be disrupted by similar kinds of plastic changes at the input stage of oculomotor control. To find out, the magnetic-field search-coil technique was used to record 2 dimensional eye movements of 3 kittens reared from birth in an environment illuminated about 8 times per sec by a 4 microsec strobe flash. During strobe rearing there was surprisingly little change in oculomotor performance. Specifically, the frequency, velocity and trajectory of saccades were normal. Optokinetic nystagmus was also normal as evidenced by slow phase eye velocities that were approximately 90% of drum speed for speeds below 15 deg arc/sec. In addition, smooth pursuits, which can be elicited only with difficulty in normally reared cats, could also be obtained from strobe reared animals. Consequently, the cells providing the retinal velocity signals for these visually guided smooth movements are not modifiable by lack of experience with continuous motion. However, strobe rearing dramatically altered the animal's ability to fixate stationary objects. In particular, fixation, which is quite stable in normal cats, is characterized by a conjugate pendular nystagmus in strobe reared animals. This nystagmus is large (about 3 deg arc peak to peak amplitude), fast (4 to 5 Hz), always seen during fixation in either strobe or continuous illumination and frequently seen in total darkness. In addition, this nystagmus is superimposed on a wandering drift which is similar in the light and dark. These results show that experience with continuous retinal image movement is essential for the development of accurate fixation in the cat.

470 BRANCHING AXONS TO "FUNCTIONALLY INDEPENDENT" MUSCLES IN THE CAT OCULOMOTOR SYSTEM. W. F. Crandall, Jr.,* J. S. Wilson* and S. J. Goldberg (SPON: P. Bach-y-Rita). Smith-Kettlewell Institute, San Francisco, CA 94115 and Department of Anatomy, Medical College of Virginia - VCU, Richmond, VA 23298.

The Retractor Bulbi (RB) muscles in the cat are composed of four slips inserting on the equator of the globe. Both Lateral Rectus (LR) and RB muscles are innervated by the VI cranial nerve branching to the RB muscle before entering the LR. Although the motoneurons of the RB and LR originate in the same nucleus, it is generally assumed that these muscles are functionally distinct. The RB is thought to be protective in producing a reflexive globe retraction allowing the nictitating membrane to sweep over the globe. The LR is the primary muscle involved in lateral eye movement. Such a muscle system would require completely separate innervation. Contrary to this view we found that in many cases intracellular stimulation of abducens motoneurons resulted in simultaneous contraction of several muscles in the abducens system.

Multiple motoneuron stimulation may be precluded for the following reasons: a) Standard intracellular techniques were employed, b) Intracellular threshold activation of all muscles for a multiple muscle unit occurred simultaneously, c) Conversely, termination of an induced spike due either to motoneuron injury or electrode displacement resulted in simultaneous termination of muscle contractions, d) High current stimulation of these units produced multiple spikes of equal amplitude along with an increase in muscle force output, however, no new muscles were brought into contraction by the higher currents.

Of the 56 units studied in three experiments, stimulation of 44 (79%) of these units resulted in contraction of more than one muscle in the abducens system. Co-contraction of the LR and slip(s) of the RB were seen in 8 (14%) of these units. In each of these eight cases only the lateral two retractor slips were found to contract. This synergistic behavior of the lateral muscles suggests an involvement of the retractor bulbi in normal rotary eye movements.

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471 ANATOMY AND PHYSIOLOGY OF IDENTIFIED INTERNUCLEAR NEURONS IN THE ABDUCENS NUCLEUS OF THE CAT. J. Delgado-García*, R. Baker, S.M. Highstein and R. Maciewicz*, Dept. Physiol., NYU Med. Ctr., New York, N.Y. 10016 and Dept. Neurosci., Kennedy Ctr. for Research, Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Injection of HRP into the oculomotor complex has suggested the presence of a population of non-motoneuronal, internuclear (Int) neurons within the abducens (Abd) nucleus. Electrophysiological studies have demonstrated that Int neurons respond like Abd motoneurons (Mns) to orthodromic vestibular and reticular inputs. The Int neurons have been shown to terminate on medial rectus (MR) Mns exclusively in an excitatory fashion. We have further characterized the Int pathway by placing HRP in the cut axons of the ascending MLF and intracellularly injecting HRP in antidromically identified Int neurons. Reconstructions of the Abd nucleus have shown that Int neurons have a morphology and distribution similar to Abd Mns. In addition, the axons of all Int neurons cross the midline at the level of the Abd nucleus with no observable axon collaterals and ascend in the dorsal medial part of the contralateral MLF. To identify and compare Int and Abd Mn activity in the alert cat stimulating electrodes were placed in the contralateral MR subdivision of the oculomotor complex and on the ipsilateral Abd nerve. Of 47 neurons antidromically isolated in the Abd nucleus, 18 were activated only from MR stimulation and 29 from only Abd nerve stimulation. As determined by recording EOG's from each eye, Int activity was found to be qualitatively identical to that of Abd Mns during all horizontal conjugate eye movements. Quantitatively the two populations of neurons were markedly different. Typically, eye position thresholds were lower and maximum firing frequency higher for Int neurons than Abd Mns. Plots of eye position vs. firing frequency for Int neurons showed a steep, non-linear increase in contrast to the linear relationship exhibited by Abd Mns. Modulations of Int neurons preceded saccadic eye movement in the ON and OFF direction by an average of 10 and 20 msec, respectively. During monocular eye movements Int activity closely paralleled changes in only contralateral eye position and velocity profiles. Int firing frequency during 2-4° vergence movements was not altered following the change in eye position. In contrast, Abd Mn firing was modulated during all vergence and versional movements. From this data, we propose that vergence movements are produced by a separate supranuclear input to MR Mns. We infer that horizontal burst-tonic fibers recorded in the MLF are axons of Int neurons from the Abd nucleus. Thus, we conclude that they are the source for relaying conjugate horizontal eye movement signals to the contralateral MR Mns for saccades and fixation. (Supported by EY-01074, EY-10003 and EY-10670).

472 TECTAL CONNECTIONS WITH THE EXTRAOCULAR MOTOR NUCLEI. Stephen B. Edwards and Craig K. Henkel. Dept. Anat., Sch. Med., Univ. of Va., Charlottesville, VA 22901.

Direct and indirect projections from the superior colliculus to the extraocular motor nuclei were studied using the orthograde autoradiographic tracing method, the retrograde HRP technique, and Golgi methods. After injections of ^{3}H-leucine into the superior colliculus, transported label was found either within the extraocular motor nuclei or in nuclear regions directly adjacent to them. Within the oculomotor region, a focus of label was present in the central gray matter directly overlying the nucleus all along its rostrocaudal extent. This label was always more dense than that in the interstitial nucleus of Cajal or the nucleus of Darkschewitsch-purported accessory oculomotor structures. No label was present in the oculomotor nucleus itself. Golgi material revealed that dendrites of many oculomotor cells extend dorsally into this tectal terminal zone, thus providing the opportunity for direct tecto-oculomotor contacts. Horseradish peroxidase injected into the abducens nucleus produced retrograde labeled neurons clustered specifically in the tectal terminal zone overlying the oculomotor nucleus. This finding suggests that cells in this zone provide indirect tectal input to the abducens nucleus. Within the trochlear region, isotopic label transported from the colliculus was focused in a cell group directly ventrolateral to the trochlear nucleus. The projections of this labeled cell group were not studied. Within the abducens region, transported label was concentrated mostly in the reticular formation directly ventral to the nucleus. In six cases, however, label was present within the nucleus itself, though it was always sparse. The presence of this label is strong evidence for a direct, though small, tecto-abducens connection. Tectal labeling in the extraocular motor regions was present only after isotope injections in the deep gray layers of the colliculus and was heaviest in those cases with rostrally placed injections. Label in the abducens and trochlear regions was densest after injections into the stratum griseum intermedium, and thus appeared to have a preferential origin from this layer.

473 SINGLE UNITS IN THE CAT THAT PAUSE FOR SACCADES. C. Evinger,* C.R.S. Kaneko and A.F. Fuchs (SPON: T. Kennedy). Dept. Physiol and Biophysics, and Regional Primate Research Center, Univ. Wa., Seattle, WA 98195.

Although both anatomical and electrophysiological studies have begun to reveal the connectivity of brainstem oculomotor structures in the cat, very little is known about the functional properties of these neurons. In order to examine the roles of brainstem neurons in eye movements, the activity of single units in the region of the abducens nuclei were recorded in cats trained to a visual tracking task. This study deals with one group of neurons, the pausers, found clustered around the midline, anterior to the abducens nuclei that appear to be significant in the generation of saccadic eye movements.

Pausers are tonically active and cease their discharge just prior to and during saccades. The pause in firing begins 12-20 msec before the onset of the saccade. The pause duration usually lasts longer than saccade duration, and pause duration increases linearly with saccade duration. In the majority of the units the tonic firing level varied between 50 and 160 spikes/sec and was not related to eye position.

Pausers are also modulated by visual input. If the cat fixates a stationary target surrounded by a visual noise background sinusoidally oscillating in the horizontal plane, the cells' firing rate is modulated such that minimal firing frequency occurs during maximal background velocity during both left and rightward background movement. Retinal slip velocities of 20-40 deg/sec produced maximal unit modulation but retinal slip velocities in excess of 100 deg/sec also modulated pauser activity.

Microstimulation at the site of pauser recordings specifically inhibited both horizontal and vertical saccades. Continuous 30μA pulse trains (300 Hz, bipolar, .1 msec square wave) prevented all saccades but did not interrupt smooth eye movements in response to a sinusoidally modulated optokinetic target. Brief (10-50 msec) 20μA pulse trains triggered by the onset of a saccade interrupted that saccade for a period equal to the stimulus duration.

474 RESPONSES OF NECK MOTONEURONS TO STIMULATION OF THE INTERSTITIAL NUCLEUS OF CAJAL. K. Fukushima, N.G. Pitts and B.W. Peterson. Rockefeller Univ., New York, N.Y. 10021.

The interstitial nucleus of Cajal (INC) has been considered to be particularly important in vertical and rotatory gaze control. Since both head and eye movements contribute to the shifts of gaze observed when the region of the INC is stimulated (Hassler & Hess, 1954), it seemed reasonable to expect that interstitiospinal fibers might establish synaptic connections with neck motoneurons. To detect such connections, we have studied the responses of antidromically identified neck motoneurons to stimulation of the INC in cats under chloralose anesthesia. Multi-electrode arrays consisting of 5 electrodes, 1-1.5 mm apart in a mediolateral direction, were inserted into the mesencephalon so that the central electrodes recorded antidromic responses (0.6-8 msec latency) of INC neurons to stimulation at the C_4 spinal segment. Histology showed that these electrodes were in the INC. The other electrodes were used to delimit the effective area of stimulation. 100μA, 100μsec monopolar pulses were applied to the electrodes and responses were recorded intracellularly from neck motoneurons.

Stimulation within the INC evoked EPSPs at latencies of 0.7-1.4 msec in many neck motoneurons. If 0.5 msec is allowed for conduction time, these latencies suggest monosynaptic connections. The effective area for evoking these EPSPs corresponded well to the INC and thresholds were often 10-50μA. No monosynaptic PSPs were evoked by electrodes located 1.0 mm or more from the INC or interstitiospinal tract. Monosynaptic EPSPs were evoked from the ipsilateral INC in 27/27 biventer-cervicis-complexus (BCC), 10/15 splenius (SP) and 4/10 trapezius (TR) motoneurons and from the contralateral INC in 6/11 BCC, 1/10 SP and 2/7 TR motoneurons. INC stimulation also evoked later, presumably disynaptic, EPSPs in BCC and TR, and mixed EPSPs-IPSPs in SP motoneurons. The excitatory responses evoked by INC stimulation were considerably greater in BCC motoneurons than in TR or SP motoneurons.

Simultaneous stimulation of the INC and medial vestibular nuclei or ponto-medullary reticular formation produced monosynaptic PSPs that exhibited simple summation. Hence monosynaptic EPSPs evoked by INC stimulation do not appear to be mediated by bifurcating vestibular or reticular axons. Thus our results indicate that the INC directly excites many neck motoneurons. The pattern of consistent excitation of dorsal neck extensors and weaker, mixed excitation and inhibition of lateral flexors and muscles with a more complex action is consistent with the view that the INC causes movements resulting in vertical and rotatory gaze shifts. Supported in part by Grants NSF BMS 75 00487 and NIH NS 02619. Hassler, R. & Hess, W.R. Arch.Psychiat.Neurol.192(1954)488.

475 ANATOMICAL ORGANIZATION OF CAT ABDUCENS MOTONEURONS RELATED TO SINGLE MOTOR UNIT MECHANICAL CHARACTERISTICS. S.J. Goldberg and H.P. Clamann. Depts. Anat. and Physiol., Med. Coll. of Va.-VCU, Richmond, VA. 23298.

There is histological and physiological evidence indicating diverse types of extraocular muscle fibers. Electrophysiological studies have shown functional differences in extraocular motoneurons related to impulse firing rates. Although it is generally accepted that most motoneurons in a particular nucleus can participate in all types of eye movement (saccades, pursuit and fixation), individual cells may exhibit firing frequencies better correlated with specific types of eye movement. This study was undertaken to determine if abducens nucleus (ANu) - lateral rectus muscle (LR) motor units displayed any features which might further the clarification of extraocular functional characteristics.

Cats were anesthetized and the abducens nerve was stimulated within the brain stem. ANu motoneurons were identified by their antidromic responses to VIth nerve stimulation. Single motor units of the LR were activated with short current pulses delivered through an intracellular micropipette penetrating a single motoneuron, and the mechanical responses in the LR were recorded with a sensitive strain gauge attached to the freed LR in the orbit.

A total of 30 ANu motoneurons were studied intracellularly, but all mechanical parameters of each motor unit could not always be examined. We found that the motor units had a wide range of twitch tensions (1.3 - 50 mg.), but units with contraction times slower than 6.5 msec. had a tendency towards twitch tensions of less than 15 mg. Motoneurons encountered deeper than 1.7 mm. beneath the floor of the IVth ventricle also tended to feed motor units with twitch tensions of less than 15 mg. But the most striking feature was a clear trend from fast (3.5 msec.) to slow (10.3 msec.) twitch contraction times as we penetrated motoneurons from dorsal to ventral in the ANu. Further investigations are needed to understand the possible functional significance of this motor unit organization.

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476 EYE AND HEAD MOVEMENTS EVOKED BY STIMULATION OF CAT SUPERIOR COLLICULUS. D. Guitton, A. Roucoux+ and M. Crommelinck+ (SPON: M. Meulders). Univ. Louvain, B - 1200 Brussels. Belgium.

The lower layers of the superior colliculus (SC) contain a motor map which, in monkey, is in register with an overlying retinotopic projection. Saccades evoked by stimulating a point in the deeper layers are coded in retinal coordinates: they have constant amplitude and direction, independent of the eye's initial position in the orbit. In cat, the situation is less clear : 1) eye movements, that are goal directed with respect to the head, have been reported[1,2]; and 2) the sensory map covers more than 60° of visual field whereas eye movements are limited to 25° from central gaze position. These facts suggest that cat SC might also code head movements. Monopolar stimulation of the deep layers of the SC was performed in chronic cats with head fixed or free. Currents ranged between 5 and 15 µA. Eye and head movements were accurately recorded (to .1°) using the search coil in magnetic field technique. Results differed according to the location of the stimulated site with respect to the following zones of the overlying retinotopic map. Zone 1 (approximately the central 12°). With head fixed, evoked saccades with about 25 msec latency were retinotopic and similar to those observed in monkey. With head free, a slow head movement accompanied in the same direction the evoked saccade with similar short latency. This result differs from monkey. Zone 2 (about 12 to 25°). With head fixed, stimulation of a single point in SC brought the eye to a single point in the orbit: saccades were goal-directed. With head free, the evoked saccades were slightly preceded by a rapid (100-200°/sec) head movement. The direction of the evoked head movement was constant and independent of the head's initial position. Zone 3 (far periphery). With head fixed, centering eye movements were evoked. When the head was released, the evoked head movements were very rapid and goal directed with respect to the body. Results suggest that in cat, unlike in monkey, 1) the oculomotor and visual maps are not necessarily in register and 2) the SC is directly implicated in head motor control.

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2. STRASCHILL M. and RIEGER P. Brain Research 59 (1973) 211-227.

477 CANCELLATION OF THE VESTIBULOOCULAR REFLEX DURING ACTIVE AND PASSIVE HEAD MOVEMENTS IN THE NORMAL CAT. G.M. Haddad* and D.A. Robinson. Dept. Ophthal., Sch. Med., Johns Hopkins, Baltimore, MD 21205.

Circuit diagrams of the oculomotor system are frequently developed by correlating functional performance to single cell activity in the alert monkey and explaining this correlation by structural interconnections found in the depressed cat. However, common laboratory experience suggests that normal oculomotor performance in the cat may be quite different from that of monkey. This could cause serious errors in extrapolating cat anatomy to monkey behavior. This research addresses two questions in the cat for which we have answers in the monkey: 1) do saccades (quick phases) and vestibular velocity commands just add during vestibular nystagmus and 2) when cats track objects with their heads do they cancel their vestibulooocular reflex (VOR)?

Three normal adult cats were studied. Eye movements and head movements were measured by the technique of coils moving in a magnetic field. The gain of their VOR (slow phase eye velocity/ head velocity) in the dark was normal (e.g., .92).

Peak velocities (eye in head) of saccades ranging from 5-15 degrees were measured with the head stationary, the head passively rotated (~ 100 deg/sec) and the head actively moving (~ 50 deg/sec). Saccade velocity was always higher during either active or passive head movements than with the head stationary. For example, the cats typically made 10 deg saccades, head stationary, whose average peak velocity was 86 deg/sec. The same amplitude saccades during active 50 deg/sec head movements had an average peak velocity of 122 deg/sec and during passive rotation at 100 deg/sec, the average peak velocity was 142 deg/sec. All cats showed the same effect, i.e., saccades during head movements were faster than saccades with the head stationary. This result implies that saccadic and vestibular velocity commands do not just add. If they did, one would expect that saccades made during head rotation would be slower than saccades made with the head stationary. We found the opposite effect.

We also measured intersaccadic gaze velocities (eye velocity in space) during active and passive head movement as the cats looked about the room. Cats used their VOR to keep intersaccadic gaze velocity essentially zero despite head movements as fast as 90 deg/sec. Mean gaze velocity for one cat on one day was 0.6 deg/sec, $\pm$ 5.7 (s.d.). However these cats were also able to cancel the VOR during head tracking (of food). During tracking the ratio of gaze velocity to head velocity (which would be 1.0 for perfect cancellation) was about 0.7 (0.6 s.d.) for head velocities ranging from 3-60 deg/sec. Gaze velocity matched or was higher than head velocity about 40% of the time.

478 CELLS RELAYING LABYRINTHINE SIGNALS TO THE IIIRD NUCLEUS THROUGH THE BRACHIUM CONJUNCTIVUM IN THE RABBIT. S.M. Highstein, M. Yamamoto*, I. Shimoyama*, R.J. Maciewicz* and A. Steinacker*. Albert Einstein Col. Med., Bronx N.Y., Univ. of Tokyo; Faculty of Med., Tokyo, Japan.

Excitatory signals from the labyrinth to oculomotor neurons are conveyed by the Medial Longitudinal Fasiculus (MLF) as well as by the Brachium Conjunctivum (BC). The BC pathway relays anterior canal (AC) excitation to the ipsilateral superior (iSR) and the contralateral inferior oblique (cIO) extraocular muscles. However, the cells of origin of this BC mediated excitation are in question. To elucidate the location of these cells, a morphophysiological approach was undertaken.

The brainstems of anesthesized paralyzed rabbits with bilaterally transected MLFs were systematically tracked with a recording microelectrode during stimulation of the AC and IIIrd nucleus. Locations of units ortho- and antidromically activated were marked with fast green FCF iontophoresed from recording pipettes and later plotted on reconstructed brainstem sections.

Horseradish peroxidase (HRP) was iontophoresed into the IIIrd nuc. of rabbits with intact brainstems or with lesions of the MLF or BC. After 24 hours animals were sacrificed and brainstem sections reacted with diaminobenzidine and H_2O_2. Cells labeled with HRP reaction product were plotted on photographs of brainstem sections. Morphological and physiological results suggest that there is a group of cells in the dorsal portion of the superior vestibular nucleus which project through the BC to the IIIrd nuc. These cells presumably mediate the excitatory signals from the AC to the motoneurons innervating the iSR and cIO extraocular muscles. Further, cells in the center of the superior vestibular nuc. project to the IIIrd nuc. via the MLF and apparently mediate inhibitory labyrinthine signals to ipsilateral oculomotor neurons.

To elucidate other components of the BC pathway the Y group and lateral cerebellar nucleus were tracked during whole VIIIth nerve and IIIrd nuc. stimulation. Y group and lateral nuc. cells were antidromically activated from IIIrd nuc. but required multiple VIIIth nerve shocks for orthodromic activation.

To confirm these pathways superior vestibular, lateral nuc. and Y group cells were injected intracellularly with HRP and their axonal and dendritic projections traced.

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BURSTING EYE-MOVEMENT RELATED NEURONS RECORDED IN THE BRAINSTEM OF THE ALERT TRAINED CAT. C.R.S. Kaneko, C. Evinger*, and A.F. Fuchs. Dept. Physiol. & Biophys. and Regional Primate Res. Ctr., Univ. Washington, Seattle Wa. 98195.

In order to provide information on the possible function of cat ocular motor structures presently being studied by intracellular and anatomical techniques and to provide some support for the untested assumption that feline and simian ocular motor systems are similarly organized, we recorded from single midline pontine neurons in the alert cat. We surveyed the types of eye-movement neurons and mapped their anatomical locations. Quantitative characterization of the firing parameters of these neurons correlated with eye-movement parameters was employed to discern possible causal relationships. This report deals with neurons in the region of and anterior to the abducens nucleus which fire a burst of action potentials for fast eye movements.

Extracellular recordings from cats trained to track moveable visual targets have revealed several types of bursting neurons. Burst units may be divided into those that have a tonic level of firing and those that do not. The former may be subdivided into medium-lead and long-lead bursters with latencies from first spike to eye-movement of 6 to 14 and 15-30 msecs respectively. Burst frequencies were as high as 350 spikes/sec and durations varied from 1 spike to several hundred msecs in length. The former group tended to be located more anteriorly than the latter.

The on-direction (angle) of eye movement associated with maximal firing of the neuron) varied from purely ipsilateral to omni-directional for either medium-lead or long-lead bursters. Unlike analogous neurons in simian brainstem, these units were not uniquely related to fast eye movements but might fail to fire for on-direction movements or might fire in the absence of an eye movement. We have not recorded from units uniquely related to eye movements, nor have we found burst units which burst following fast eye movements as has been demonstrated for the monkey.

Burst parameters such as average frequency or number of spikes are significantly correlated with horizontal components of saccade parameters such as amplitude and size but not with vertical components.

Neurons showing a tonic firing were either units whose tonic level was eye-position sensitive or was not. The former will not be considered here. The latter appear to be an eye-movement related neuron unique to the cat, although they are rare. These units burst for fast eye movements in a large range of directions. Such units are apparently akin to omni-pausers but fire a burst instead of pausing for fast eye movements. A detailed analysis of the extent of this similarity is under way.

481 AN UNEXPECTED EFFECT OF EXPECTATIONS ON SLOW OCULOMOTOR CONTROL Eileen Kowler*, Robert M. Steinman, and Barbara J. Winterson*. Dept. Psychol., Univ. of Md., College Park, Md. 20742.

Eye movements were recorded (sensitivity < 1') while subjects used saccades to track square-wave motion of a bright point moving in darkness. They tracked for 20 seconds at a particular frequency (0.25, 0.375, or 0.5 Hz) with amplitude (p-p) set either at 12', 25', 50' or 100'.

Anticipatory drifts were observed in well over half of the tracks. These drifts moved the eye smoothly in the direction of the expected target step before the step occurred. Such anticipatory drifts were common with both vertical and horizontal target steps. They began long before the target stepped—as much as one-half second. Their velocities were high, frequently 40'/sec and faster. These anticipatory drifts did not depend on initial eye position or directional idiosyncracies of slow control.

Anticipatory drifts are "natural" eye movements in the sense that they were observed in all subjects beginning with the first 20 second trial and continued throughout a long series of measurements. N.B. None of the subjects could make directed smooth eye movements in the absence of an afterimage or smoothly moving target. However, they all made anticipatory drifts when they expected a target step.

Much to our surprise anticipatory drifts did not go away when the target stepped on a highly structured stationary background. There were more surprises when we attempted to find out whether the expectation of the target step or the expectation of making a saccade caused the eye to drift in the direction of the expected target step. We found that both expectations were essential. Anticipatory drifts were largely abolished when subjects were required to keep the eye in place while the target stepped back and forth. They were also largely abolished when the stepping point was replaced by 2 stationary points and the subject was required to saccade from one to the other in time with a periodic auditory signal.

Anticipatory drifts are not restricted to square-wave target motions. Predictable ramps (2.2°/sec and 4.4°/sec) often have negative latencies of several hundred msec. Unpredictable ramps do not.

Our observations, admittedly mysterious as well as unexpected, have implications. They show that expectations can be a powerful input to the slow oculomotor subsystem. This is news. They also raise questions about the independence of the fast and slow subsystems because anticipatory drifts are activated only when saccades will be made.

480 CRITICAL STRUCTURES FOR DOWNWARD GAZE IN MONKEYS. Detlev Kömpf*, Tauba Pasik and Pedro Pasik. Dept. of Neurology, Mount Sinai School of Medicine, CUNY, New York, N.Y. 10029.

Only two patients with isolated downward gaze palsy can be found in the literature, and both had widespread bilateral pathology in the medial thalamus, subthalamus and rostral midbrain. In the attempt to delineate the critical region for downward eye movements, ten monkeys (M. mulatta) were stimulated through bipolar concentric electrodes placed stereotactically on each side of the midline under light barbiturate anesthesia. Stimuli consisted of a single 1-3 sec train of 0.5 msec pulses at 250 Hz. Stimulations were made unilaterally, and bilaterally in homotopic points every mm between the H+9 and the H-2 planes. The A planes between +5 and +13 were explored from 1.5 to 6 mm from the midline. At the completion of each experiment, bilateral or unilateral electrolytic lesions were made in placements where stimulation had elicited downward eye movements.

Bilateral simultaneous stimulations caused straight conjugate downward gaze from an extended area in the ventral mesodiencephalic junction from A+7 to A+11, up to 6 mm from the midline, and from H+3 to H+1. Unilateral stimulation of these points resulted in straight down, oblique down and contralateral, or no deviations at all. Bilateral lesions caused impairments of vertical gaze which were more pronounced in, and in one animal were restricted to the downward direction. Oculocephalic reflexes, however, were preserved. Electrooculograms of optokinetic nystagmus with stimuli moving in the horizontal, vertical or oblique planes, and of vestibular nystagmus elicited by caloric and turning tests, showed marked decrease or absence of responses in the direction of the defective gaze. Oblique optokinetic stimulation elicited a perverted response in the horizontal plane. Bilateral simultaneous warm caloric irrigations with the monkey in the erect position produced a strong upward deviation of the eyes. In some cases, there was also a shimmering or weak downward nystagmus, which, however, never reached below the horizontal meridian. Double cold irrigations failed to produce the tonic deviation downward in most animals and elicited in some cases an upward nystagmus. Similar findings were obtained by turning the animals while lying on the right side. The postrotatory response after clockwise rotation was equivalent to that elicited by bilateral warm irrigation. All responses in the horizontal plane were consistently normal. Recovery occurred in all types of vertical eye movements except in rapid deviations below the horizontal meridian which were absent during the entire 3-month follow up period. A unilateral lesion had no effect. These findings indicate that both sides of the ventral mesodiencephalic junction contain critical structures for downward rapid eye movements. Aided by N.I.M.H. Grant # MH-02261.

482 SMOOTH PURSUIT TRACKING OF PERIODIC AND NON-PERIODIC TARGETS IN MAN. S.G. Lisberger, L.C. Evinger* and G.W. Johanson.* Regional Primate Research Center, Univ. Washington, Seattle, WA and Universität München, Germany.

The purpose of these experiments was to re-evaluate the human smooth pursuit system in the light of recent experiments suggesting it might not function in the same way as the velocity-servo demonstrated by Collewijn in rabbit. For periodic tracking subjects were asked to track a sinusoidally moving target at a variety of amplitudes and frequencies. Gain was computed (after Meiry) as the average peak-to-peak amplitude of 25 cycles of de-saccaded eye position (E) divided by the peak-to-peak amplitude of target position (T). When gain was plotted as a function of frequency (f) or peak target velocity ($VT=(2\pi f)T$) each amplitude of target movement formed a different characteristic with greater gain for smaller target amplitude at any given frequency. When gain was plotted as a function of peak target acceleration ($AT=(2\pi f)^2T$) all data from all frequencies (0.4 to 2.1 Hz) and all amplitudes (±5 to ±25 deg) fell along the same characteristic. Similarly, when eye velocity (VE) was plotted as a function of retinal error velocity (REV=VT-VE), each target amplitude produced a different characteristic, but when eye acceleration was plotted as a function of REV, all data points fell along the same characteristic. For non-periodic tracking, subjects were asked to track a "random walk of sinusoids" in which the probability that the target changed direction at a peak or trough was reduced to 0.5. However, 1 of each 4 cycles (on the average) was a full sinusoid and could be analysed using the techniques applied to periodic tracking. Thus this target movement greatly reduced predictability, but at the same time allowed easy analysis. Although tracking capabilities in this paradigm were only 10 to 16% of periodic values and eye position lagged target position by up to 55° deg at 1 Hz, the results were qualitatively the same as for predictive tracking. Gain was best described as a function of target acceleration and eye accelaration was better related to REV than eye velocity was. Our results suggest that one basic property of the human smooth pursuit system is that target slip on the retina causes eye acceleration. Therefore, our results support the corollory discharge model of smooth pursuit in which a neural positive feedback pathway would sustain eye velocity while visual input would cause corrective changes in eye velocity, or eye acceleration. (Supported by Grants RR00166, EY00745 from NIH, USPHS)

483 CONTROL OF CHAMELEON SACCADE TIMING. J. W. B. Mates* (SPON: J. M. Horowitz). Dept. Biology, Univ. Oregon, Eugene, OR. 97403.

African chameleons scan their environments by saccadic movements of their eyes. But saccades of left and right eyes of these lizards often occur at different times. Thus, one chameleon generates two sequences of eye movement times, one for each eye.

An automatic saccade recognition system recorded saccade time sequences from taped EOG records. Sequence durations exceeded four hours. Sequences contained several thousand saccade times each. The recognition system found all saccades with horizontal amplitudes in excess of 1°, and the system specified saccade times to within 2 msec. Ten chameleons of three species (C. dilepis, C. jacksoni, and C. hohnelii) provided more than 200,000 saccadic eye movements for analysis.

Histograms of intervals between successive saccades invariably had exponential tails. This result suggested that, following a saccade, the likelihood of a second saccade settled after about 1 sec to a constant low value per unit time (ca. 0.5/sec). I conjectured that visual environment changes would cause changes in the slopes of the exponential tails.

First-, second- and third-order statistics, taken from whole and segmented saccade sequences, confirmed that (except for the 1 sec neighborhood effect) chameleon saccade generation was a constant probability (i.e., Poisson) process. But saccade timing statistics of left and right eyes were always closely similar, despite exposure to differing left and right visual environments. Even chameleons with one or both eyes occluded did not alter the time constants of their saccade generating processes. I concluded that chameleons in static visual environments made most saccadic eye movements by a constant probability stochastic process unaffected by visual input.

484 PLASTIC ADAPTATION OF SACCADIC DYSMETRIA. Lance M. Optican* and David A. Robinson (SPON: M. H. Goldstein, Jr.). Dept. Biomed. Eng. and Ophthal., Johns Hopkins Sch. Med., Baltimore, MD 21205.

The innervation for a saccade is a pulse to move the eye rapidly against viscous drag and a step to hold the eye against the elastic restoring force. If the pulse is not matched to the step, the eye will drift exponentially at the end of the saccade. When the pulse and step match, but are both either too big or small for the retinal error, saccadic dysmetria occurs. This study shows that both types of disorder are repaired by the brain.

Trained Rhesus monkeys had the position of both eyes monitored by a scleral search coil in a magnetic field. Each eye was calibrated seperately. Under anesthesia the horizontal recti of one eye were tenotomized and the eye was patched. After a few days the muscles reattached but were weaker. With the normal eye viewing, the weak eye made saccades hypometric by about 50% and drifted with initial velocities up to 50°/sec. The patch was then switched. Over the next few days saccades of the weak eye slowly grew until they became almost orthometric and post-saccadic drift was greatly diminished. Simultaneously, saccades in the patched, normal eye became hypermetric with a drift in the opposite direction to the original drift in the weak eye. Thus, both the size of the step and the pulse had been independently adjusted to compensate for the peripheral disorder.

A mechanical gain for the eye was defined as the ratio of the change in position of the weak eye to that of the normal eye. The gain of the saccadic system was defined as the ratio of the amplitude of the saccade to the retinal error. Results indicate that mechanical gains as low as 0.4 can be compensated, and gains for the saccadic system (monitored through the normal eye) can go as high as 2.3. Drifts caused by pulse-step mismatches as high as 40% can be suppressed by independently adjusting the gains of the pulse and the step. These changes occur with an exponential time course over 3 to 5 days. If the patch is switched back so that all visual experience comes from the normal eye, the gains will revert to their original values in another three to five days.

This adaptive ability of the saccadic system can be summarized: 1) With monocular viewing, the central compensation caused the gains of the saccadic system for the left and right eyes to change together, similar to Hering's Law; 2) the gains change with a time constant of about one day; 3) the gains for leftward and rightward movements can be adjusted independently; 4) the gains for the step and the pulse of the saccadic system can be adjusted independently.

485 ADAPTIVE GAIN CONTROL IN VESTIBULO-OCULAR REFLEX OF GOLDFISH. J.O. Schairer* and M.V.L. Bennett, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y.

Unanesthetized goldfish were restrained with their heads centered in a clear cylindrical tank on a sinusoidally rotating platform. An optokinetic drum surrounded the tank. Eye position was monitored with a magnetic search coil. The velocity gain (gain = eye velocity/fish velocity) of the vestibulo-ocular reflex (VOR) was measured in the dark and in the light. Three fish were first rotated for at least 1/2 hour in the light at 0.16 Hz. with the optokinetic drum stationary in order to establish a baseline gain. In the dark the gain ranged from 0.33 to 0.55. In the light gains were higher, ranging from 0.43 to 0.66. Then the fish and the drum were rotated together in the light for six hours (except for brief periods of dark testing). The gain measured in the dark dropped with a time constant ranging from 1.3 to 2.5 hours during the first two hours. This was followed by a continued slower drop in gain to between 7 and 33% of the baseline value at the end of six hours. The fish were then rotated with the drum stationary again. The dark gain recovered with a time constant of from 0.5 to 1.5 hours. Control experiments showed the VOR did not change significantly during six hours rotation in the dark at this frequency. Large variations in the difference between vestibulo-ocular gain in the dark and in the light were seen. During the first two hours of adaptation one fish had an average difference between dark and light gains of 0.23. The drop in gain over the same time period had a time constant of 2.5 hours. The second fish had an average difference of 0.02 over the first 2 hours with the same time constant of adaptation. The third fish had an average difference of 0.17 with a time constant of 1.3 hours. It is odd that these differences in the visual effect on eye movements showed little correlation with speed of adaptation. During recovery light gain was always substantially greater than dark gain. At least 5 other fish showed similar changes in gain during less extensive experimental runs. These data extend to a lower vertebrate the demonstration of adaptative gain control in the vestibulo-ocular reflex. The relatively rapid learning exhibited here and the previously proven capacity for intracellular recording from rotating alert fish make this an attractive preparation for studying the electrophysiology of VOR gain control.

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486 DYNAMIC OVERSHOOT IN SACCADIC EYE MOVEMENTS. Lawrence Stark, Alan B. Scott*, and Robert V. Kenyon*. University of California, Berkeley, and Smith Kettlewell Institute of Visual Science, San Francisco.

Dynamic overshoot was first predicted from theoretical consideration of the time optimal control strategy for saccades (Clark and Stark, IEEE Trans. AC-20: 345, 1975). Optimal control theory developed by Pontryagan, Bellman, Smith and Athans determines the number of switchings of maximal controller signals from the order of the plant. The plant is the eyeball and eye muscle model as developed by Cook and Stark (Arch. Ophthal. 79: 428, 1968) and by Clark and Stark (Math. Biosci. 20: 191,213, 239, 1974) that features reciprocal innervation and nonlinear force velocity relationships.

Using careful recording techniques, dynamic overshoot was found in human eye movements as predicted by theory (Bahill, Clark and Stark, Exp. Neurol. 48: 107, 1975). Since dynamic overshoots are caused by neurological control signal reversals, they therefore should be experimentally observable in neurophysiological studies in man. They would be most clearly apparent with bursts of single unit EMG activity in antagonist muscle at the end of its pause for the main saccade. Preliminary results using single unit EMG recordings in a limited sample of patients do indeed show such bursts as predicted from time optimal control theory and eye movement recording results.

Although published neurophysiological studies on primates and other mammals had not shown dynamic overshoot neural signal reversals, we understand that several laboratories are now finding such controller signal patterns. This is an exciting confirmation of the ability of neurological control studies to provide interaction with neurophysiological recordings in animals.

487 OSCILLATORY EYE MOVEMENTS WITH POSSIBLE VISUAL FUNCTION IN BIRDS
Joseph Turkel and Joshua Wallman. Dept. Biol., C.C.N.Y., New York, N.Y. 10031

A characteristic of eye movements in at least some birds is the frequent occurrence of a type of movement never seen in most mammals. These movements, which were first recorded in pigeons, consist of bursts of rhythmic, large amplitude oscillations of the eyes, often associated with eye blinks. Nye (Vis. Res. 9:133, 1969) termed these movements "polishing movements" with the implication that they are involved in grooming the eye. We have investigated these oscillatory eye movements in order to determine the role of these unique ocular movements in avian vision.

Oscillatory eye movements were measured in chicks using electro-oculograms, filmed records of movement made with a light-lever technique, and eye movement records made with a magnetic coil technique. In general, the visual axis traced a series of roughly elliptical paths with major orientation in approximately the horizontal plane during the course of a burst of eye movement. The bursts of movement typically occurred synchronously in both eyes and lasted for 100-300 msec., during which the eye oscillated at a frequency of 20-30 Hz with an amplitude of up to 15°. Both amplitude and frequency were usually greatest at the beginning of an eye movement burst. Movements of the nictitating membrane were always accompanied by oscillatory eye movements; oscillatory eye movements occurred, however, about twice as frequently (20-30/min.). Further, while local anesthesia of the eye did not affect the frequency of occurrence of these eye movements, it significantly reduced the number of membrane movements.

The relation of oscillatory eye movements to visual stimulation was also explored. In a stationary visual environment these movements occurred about as often as in the dark. During optokinetic nystagmus they invariably occurred during the quick phase. We did not find a relationship between the path traced by the eye and the spatial pattern of the stimulus, either with moving or stationary stimuli.

Oscillatory eye movements appear then to at least be under some visual control in chicks in that they are triggered by visual stimulation, and not necessarily related to movements of the nictitating membrane. An additional finding which supports the idea that these oscillations are involved in visual processing is that the occurrence of bursts of eye movement are synchronized to an ongoing tectal EEG rhythm of 3-5 Hz.

488 INTERACTIONS BETWEEN THE EYES IN OPTOKINETIC NYSTAGMUS OF CHICKENS.
Josh Wallman, Joseph Turkel, David H. Eastzer*, and Mok Hin-Kiu*. Biology Dept., City College, CUNY, New York, N.Y. 10031.

In most mammals, visual pursuit is accomplished by equal movements of both eyes. Even when it is dysfunctional, as when one eye is blind or partially paralyzed, the motor output to the muscles of both eyes is equal (Hering's Law).

By recording the movement of the eyes and the head with small search coils in an alternating magnetic field, we have found that in chickens the movements of each eye are much more independent than in mammals and have an interesting relation to each other.

Both the quick and slow phases of optokinetic nystagmus occur synchronously in the two eyes. The velocity of the slow phase of optokinetic nystagmus is usually very different in each eye. If one eye is closed, the closed eye makes normal movements but with much lower velocity than the open eye. Even if both eyes are open, the velocities of each may differ by a factor of 2 or more, with the eye for which the stimulus direction is temporal-to-nasal moving at higher velocities.

The following evidence argues that these phenomena result from partially independent programming of the movements of the two eyes rather than from shifts in the proportion of motor output to the two eyes: (1) The ratio of slow-phase velocities of the two eyes depends on the stimulus velocity; (2) The slow-phase velocity of the eye moving in the temporal-to-nasal direction is less variable (lower coefficient of variation) than that of the other eye; (3) In a stimulus situation in which the stripes move in opposite directions for each eye, the bird makes normal nystagmus movements, synchronously in both eyes, but in opposite directions; (4) In a stationary environment, or in the dark, saccades are always synchronous, but are more often disjunctive than conjugate, and more often unequal than equal in amplitude.

Studies on the development of these interactions and the effect of monocular visual deprivation are in progress.

489 EYE AND HEAD MOVEMENTS TO AUDITORY TARGETS.
Douglas A. Whittington* and Marie-Claude Hepp-Reymond. Dept. Psychol., M.I.T., Cambridge, MA. 02139 and Institut für Hirnforschung, CH 2029 Zurich, Switzerland.

Rhesus monkeys were trained to direct their gaze toward hidden auditory targets consisting of single clicks or click-trains delivered through small loadspeakers located along a perimeter arc in the horizontal plane in front of the monkeys. The monkeys received a water reward when they directed their gaze to within five degrees of the target. The experiments were conducted under two conditions; one in which the monkeys' heads were restrained, and the other in which they were free to rotate in the horizontal plane.

When the monkeys' heads were restrained, and auditory targets were presented at various locations along the perimeter arc, the animals responded by making saccades toward those targets. These saccades differed from saccades to visual targets in that their latency averaged 118 ms. (n=238, σ=44 ms.), while saccades to visual targets taken during the same recording sessions had an average latency of 215 ms. (n=45, σ=46 ms.). Since the animals were not constrained to have their eyes in a specific position when each auditory trial started, the saccades elicited began from a variety of initial positions, and it was found that the accuracy of the saccades was independent of the initial position of the eye. If one accepts the concept of a retinocentric coordinate system for saccadic movements, the preceding observation argues that a remapping takes place in which the head-referenced auditory target position is remapped into retinocentric coordinates by taking into account the initial position of the eye in the orbit.

When the monkeys were allowed to move their heads as well as their eyes in attempting to fixate an auditory target, they used a strategy which appears to be the same as that used to fixate visual targets. That is, they made a saccade toward the target and, as the head began to rotate to the target, the eye performed a compensatory movement which kept the gaze on target during the head movement. This strategy was employed when the auditory stimulus was present throughout the movement as well as when the stimulus was extinguished just prior to the onset of the eye and head movement, thereby removing the contribution of auditory feedback. The most striking difference between the responses to auditory and to visual targets was that the latencies for both eye and head movements were markedly shorter for auditory targets. (Research supported by NIH grant NS09343 and NASA grant NGR 22-009-798.)

490 THE EFFECT OF PULVINAR AND LATERAL POSTERIOR NUCLEUS STIMULATION ON CELLS OF THE ABDUCENS NUCLEUS.
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Electrical stimulation of the pulvinar-lateral posterior nucleus (Pul-LP complex) in the alert cat produces contraversive, conjugate, saccadic eye movements. The exact nature of Pul-LP complex's input and pathway to the abducens nucleus is unknown. The purpose of this study was to determine the intracellular response characteristics of abducens cells to Pul-LP complex stimulation.

Cats were anesthetized during surgery and immobilized with gallamine triethiodide prior to recording. The vermis of the cerebellum was aspirated to expose the floor of the 4th ventricle. The abducens nucleus was identified by antidromic field potential responses to stimulation of the abducens nerve in the orbit. Bipolar stainless steel stimulating electrodes were inserted into the Pul-LP complex contralateral to the abducens nucleus recording site. The Pul-LP complex was stimulated with either single or trains of monopolar rectangular pulses. The location of all stimulation sites was confirmed histologically.

Preliminary results include a study of 43 motoneurons (MN's), 24 of which responded to Pul-LP stimulation. Pul-LP stimulation produced action potentials in 17 MN's with latencies generally between 15-30 ms. All latencies were measured from the first stimulus pulse. Intracellular records in six of these MN's showed EPSP's with latencies between 8-25 ms. Six other MN's also showed EPSP-IPSP sequences with EPSP latencies between 8-25 ms. Seven cells appeared to receive a purely inhibitory input. 19 MN's did not respond to Pul-LP stimulation. 11 of these were recorded intracellularly.

Five interneurons, which were orthodromically activated by abducens nerve stimulation, showed action potentials in response to Pul-LP stimulation. Their latencies were between 13-28 ms. Their threshold to Pul-LP stimulation was about half that needed to drive the MN's.

The pulvinar-LP complex appeared to have a predominantly excitatory polysynaptic input onto contralateral abducens motoneurons, although 19 cells showed no response and 6 were inhibited. Studies are in progress to determine if particular Pul-LP sites effect abducens neurons differentially and if the ipsilateral Pul-LP complex has a predominantly inhibitory input.

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FEEDING AND DRINKING

491 CHANGES IN FOOD-ASSOCIATED DRINKING FOLLOWING PERIVENTRICULAR LESIONS OF THE ANTERO-VENTRAL THIRD VENTRICLE (AV3V). Steven L. Bealer. Dept. Psychol., Univ. of Iowa, Iowa City, IA 52242.

Lesions of the periventricular tissue of the antero-ventral third ventricle (AV3V) have been shown to produce a temporary post-lesion period of adipsia and chronic drinking deficits to thirst challenges (Buggy & Johnson, Am. J. Physiol., in press). The present experiment was designed to examine the effects of AV3V lesions on meal associated water intake in the rat. Ad lib eating and drinking responses were monitored continuously for several days before and after electrolytic lesioning of the AV3V periventricular tissue. After an initial period of post-lesion adipsia, spontaneous fluid consumption returned in most animals. It was found that in neurologically intact (pre-lesion) animals, there was a significant positive correlation between the volume of water associated with a period of eating and the size of that meal. This was previously reported by Fitzsimons and Le Magnen (JCPP, 1969, 67, 273-283). However, following the lesion, after daily intakes of water had returned to pre-lesion volume, the correlation between the amount of water associated with a meal and meal size was not significant. These data indicate that ablation of AV3V periventricular tissue attenuates the rats' ability to respond with appropriate volume intakes to hydrational challenges evoked by the consumption of food.

492 FAILURE OF GLUCOSE INJECTIONS TO INHIBIT FOOD INTAKE IN WEANLING RATS WITH DORSOMEDIAL HYPOTHALAMIC NUCLEUS LESIONS (DMNL). LARRY L. BELLINGER AND LEE L. BERNARDIS. Dept. Physiol., Baylor Coll. Dentistry, Dallas, Tx 75246 and Depts. Surgery and Path., SUNY at Buffalo, N.Y. 14215.

Compared to sham operated controls, rats with DMNL are hypophagic and have a reduced body weight and linear length. In a previous study (Fed. Proc. 36:561.1977) we observed that after insulin injection rats with DMNL did not initially increase their food intake comparable to the controls. The DMNL rats were also insensitive to the food intake stimulating properties of 2-Deoxy-d-glucose. In this investigation we observed whether glucose injections would depress the food intake of DMNL rats. A total of 54 rats received bilateral lesions (1 mAmp, 8 sec.) destroying primarily the DMN while 33 rats were sham operated for injections. The animals were divided into three groups: Gr. 1, saline; Gr. 2, 1.36 mg glucose/ gm body weight and Gr. 3, 2.72 mg glucose/ gm body weight. Fifteen days after the operation food was removed from the rats. The next day the animals received, intraperitoneally, their respective injections. Food was returned and then measured hourly for the next four hours. Because the DMNL rats are hypophagic compared to sham operated animals the food intake data was normalized by expressing the amount consumed during each hour as a per cent of that animal's previous 24 hour intake. The results show the hourly intakes did not differ between DMNL and the sham operated rats after the saline injections. After the glucose injection the control rats of Gr. 2 and Gr. 3 significantly ($P<0.01$) depressed their food consumption during the first hour post injection when compared to the saline controls. Their food intake during the first hour of refeeding was also significantly ($P<0.02$) less than that of the DMNL rats receiving glucose. Compared to their saline controls the glucose injections did not depress the food intake of the DMNL rats at any measurement time. The results suggest that DMNL disrupts the animals ability to monitor internal changes in glucose that normally would effect food consumption.
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493 BODY WEIGHT AND FOOD INTAKE REGULATION IN RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS WITH PRE-OPERATIVELY REDUCED BODY WEIGHT. Lee L. Bernardis, Larry L. Bellinger and Stephen Brooks*. Depts. Surg. and Path., SUNY at Buffalo, Buffalo, N.Y. 14215 and Dept. Physiol. Baylor Coll. Dent., Dallas, Texas 75246.

Mature male rats were placed on a partial starvation regimen to gradually lower their body weight below that of ad libitum-fed rats. Bilateral electrolytic lesions were placed in the dorsomedial hypothalamic area, primarily destroying the dorsomedial hypothalamic nuclei (DMN), Sham-operated rats served as controls. Food intake, body weight and obesity index were measured for 27 postoperative days. Rats that had been fed ad libitum prior to the DMN operation (AL-DMNL) showed the previously reported postoperative hypophagia and reduced body weight. Rats that had been pre-starved to a body weight 100 gm below that of the ad libitum-fed rats prior to the DMN operation (PS-DMNL) showed a significant increase in food intake only on the first postoperative day, whereupon their food intake gradually decreased to a constant level by the 10th postoperative day. At this point, and from then on, it coincided with that of the AL-DMNL rats. The body weight of the PS-DMNL rats increased steadily from the day of operation, but their obesity index was below that of the controls. Since both PS-DMNL and AL-DMNL rats had similar food intakes from the 10th day on while the body weight of the PS-DMNL rats was at that time lower than that of the AL-DMNL rats it is suggested that the postoperative food intake changes are not an active process to adjust the weight to a new set point, as has been demonstrated for the rat with lateral hypothalamic lesions. It is concluded that the lowered set point in both PS-DMNL and AL-DMNL rats is determined by the consequences of the lesions and not by the previous body weight. It is postulated that the DMN lesions have lowered the set point for both food intake and body weight, which appear in homeostatic synchrony. The above data speak for an otherwise "normal" rat that is, however, reduced in size. Possibly, then, the DMN lesion has lowered food intake and body weight set point simultaneously so that the animal is now capable of subsisting on smaller amounts of substrate. The lack of endocrine (growth hormone, insulin), substrate (glucose, lactate, pyruvate, acetoacetate, triglyceride, cholesterol) and metabolic (glucose and palmitate oxidation and incorporation and gluconeogenesis), and the ability to compensate for additionally supplied calories seem to support this hypothesis.
GM15768, NIH

494 LIMITED DAILY ACCESS TO FOOD DRIVES--BUT FAILS TO ENTRAIN--CIRCADIAN RHYTHMS IN RATS. Ziad Boulos*, Alan Rosenwasser* and Michael Terman* (SPON: Parvati Dev, MIT). Dept. Psychol., Northeastern Univ., Boston, MA 02115.

Rats maintained under constant light with free access to food and water showed free-running circadian rhythms of feeding and drinking with periods greater than 24 h. Access to food was then limited to 4 h per day over 3-8 wk, while water remained continuously available. Under the feeding schedule the drinking rhythm rapidly assumed 24-h periodicity, with most of the animals' daily water intake occurring during the feeding segment. When food was subsequently made freely available both feeding and drinking assumed their original free-running periods. However, the phase of these free-running rhythms was not determined by the phase of the preceding food-access schedule. Rather, the rhythms assumed the phase they would have shown had they been free-running throughout the feeding schedule. Under true entrainment (as with light-dark schedules), free-running rhythms would be expected to assume an initial phase set by the preceding cyclic environmental agent. The results indicate that the daily feeding schedule controlled the overt phase of the feeding and drinking rhythms, but did not alter the free-running characteristics of the underlying circadian pacemaker(s). The 24-h periodicity shown during the feeding schedule would seem to result from a temporary uncoupling of the behaviors from their pacemaker(s), allowing ingestion to be passively driven.

495 THE EFFECT OF SUPRACHIASMATIC NUCLEUS LESIONS ON CIRCADIAN RHYTHMS IN THE SQUIRREL MONKEY (Saimiri sciureus). C.A. Fuller, F.M. Sulzman*, M.C. Moore-Ede*. Dept. Physiology, Harvard Medical School, Boston, MA 02115.

The suprachiasmatic nucleus (SCN) of the hypothalamus is postulated to be a key element in the neural control of circadian rhythms in mammals. Studies in nocturnal rodents have demonstrated there is a direct retinal-hypothalamic tract (RHT) from the retina to the SCN and that lesions which destroy the SCN result in loss of organization of circadian rhythms within the animal.

We have studied the role of the SCN in the circadian timing system of the squirrel monkey, a diurnal primate with a documented RHT leading to the SCN. This primate offers particular advantages in that the circadian timing system is well documented and we have the capability to measure circadian rhythms in multiple variables simultaneously (Moore-Ede, et al., Am J Physiol 232: R31-37, 1977). Unrestrained squirrel monkeys were maintained in an isolation chamber where environmental lighting, temperature and sound were controlled. Intact animals confined all of their active behavior to the lights-on portion of a 24-hr light-dark cycle (LD 12:12 hr, 600:<1 lux). Upon being placed into constant light (LL 600 lux), each of these rhythms persisted with a free-running period of ∿25 hrs.

Bilateral electrolytic (4 mA, 15 sec) hypothalamic lesions were located in the SCN using stereotaxic coordinates confirmed by X-ray visualization of the third ventricle using a radio-opaque dye (Conray) infused into the ventricles. The mean level of the behavioral variables did not change after the lesion. In LD there was a less precise phase control of the rhythms. However, most activity was still confined to the lights-on portion of the cycle, with greater phase control than seen in similarly lesioned rodents. After 8-hr phase-delays of the LD cycle the animals took ∿3 times longer to resynchronize with the new LD phase than they did before the lesions. In LD and LL there was a large reduction in the circadian amplitude as measured by 24-hr sine wave fitting. Visual inspection of the record also showed a marked alteration in the circadian organization of these variables. This preliminary evidence indicates that the SCN plays a key role in the multioscillator circadian timing system of the squirrel monkey. (Supported by grants from the Proctor Fund, NASA NAS9-14249, NSF PCM76-19943 and NIH GN-22985).

496 EFFECTS OF VAGOTOMY ON FEEDING FOLLOWING GLUCOSE INFUSION. Paula J. Geiselman, James R. Martin, and Donald Novin. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Female rabbits were duodenally cannulated and subdiaphragmatically vagotomized (vagx). Additional animals were cannulated but not vagotomized. Subsequent to either 0-hour or 24-hour food-deprivation conditions, animals were intraduodenally infused with either .9% saline or a solution of 5%, 10%, or 20% glucose (10 ml/3 kg BW). Amount of food ingested and meal patterns were monitored for the following three-hour period.

Cumulative food intake at 1, 2, and 3 hours postinfusion indicated that, after 24-hour food-deprivation, neither intact nor vagx rabbits showed suppression of feeding in response to glucose infusions. When free-feeding, however, 5% glucose suppressed cumulative food intake in the intact rabbit at 2 and 3 hours postinfusion (p's < .01). The 10%-glucose solution was ineffective in the intact rabbit. The 20%-glucose infusion produced suppression when measured at 2 hours postinfusion (p < .05), although the effect at this concentration may have been due to toxicosis. In contrast, free-feeding vagx rabbits showed no suppression of food intake to any concentration of glucose. Furthermore, the vagx rabbits showed only a 3.7-minute latency to the first meal following infusion of 5% glucose, while intact subjects showed an 18.0-minute latency. The initial satiety ratio (number of minutes to next meal/meal size) for the vagx rabbits after 5%-glucose infusion was only 4.9 as compared with 7.4 for the intact animals.

Thus, the above results provide evidence that 5%-glucose solution administered into the duodenum of a free-feeding, intact rabbit is effective in the suppression of short-term food intake; but a simple monotonic relation between glucose amounts and degree of feeding suppression was not found. While glucose suppression of feeding was again demonstrated to be vagally dependent, the mechanism of glucose-induced suppression is complex and effects on pancreatic secretion may be involved in an explanation of the effects of different glucose loads.

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497 EFFECTS OF CHRONIC INTRAVENTRICULAR ADMINISTRATION OF ANGIOTENSIN II ON DRINKING BEHAVIOR AND BLOOD PRESSURE. Robert J. Gronan & Donald H. York, Dept. of Physiol., Sch. of Med., Univ. of Missouri, Columbia, Mo. 65201.

Angiotensin II (AII) stimulates drinking behavior and appears to increase blood pressure and ADH release through actions on the central nervous system. Evidence has been presented that these actions of AII are mediated at sites adjacent to the brain ventricles. Since an increase in plasma AII is associated with the development of certain forms of hypertension, increased levels of AII acting on the brain over a period of days may be involved in this process. It was thus of interest to determine the effects of long term infusion of AII intraventricularly (IVT). This procedure has been facilitated by the introduction of implantable osmotically driven minipumps (Alza).

AII was delivered continuously into the right lateral ventricle of Sprague-Dawley rats (400-500 g) using subcutaneously implanted osmotic minipumps connected to ventricular cannulas with PE tubing. AII was delivered in a volume of 1 µl/hr of saline vehicle at a dose of 10 ng/hr for one week. Water consumption and urine output were measured daily. Blood pressure was determined once daily in unanesthetized animals via catheters chronically implanted in the abdominal aorta. Water consumption was greatest during the second day of IVT AII infusion, reaching 209 ml/day, as compared with a mean of 44 ml/day in rats infused with AII subcutaneously (SC), and 38 ml/day prior to AII infusion. After the second day, water consumption decreased to approximately 160-180 ml/day with continued IVT infusion. Urine output followed a similar pattern. Blood pressure showed little change during the first two days, and increased after the second day a modest 10-15 mm Hg in rats receiving AII IVT. Blood pressure in the AII SC group did not differ from the pre-infusion level of 100 mm Hg. These results demonstrate a substantial increase in drinking behavior with continued IVT administration of a low dose of AII, and the possible development of tolerance or some other adaptive process, and a delayed, but small, increase in blood pressure with prolonged infusion into the lateral ventricles. (Supported by PSH HL 07094).

498 INGESTIVE REFLEXES OF THE SUCKLING RAT. W. G. Hall and Jay S. Rosenblatt.* North Carolina Dept. Mental Health, Raleigh, NC 27611 and Inst. Animal Behav. - Rutgers, Newark, NJ 07102.

The infant rat has two distinctly different ingestive reflexes. One of these is a rapid form of intake that occurs when the suckling pup receives milk-deliveries from its mother's nipple, and during which it makes a dramatic extensor response but no mouthing movements. The pup's other ingestive reflex, obvious lapping and swallowing, can be experimentally elicited by placing liquid diet in the pup's mouth when it is not suckling. The relationship of these two ingestive responses has been investigated using an intra-oral cannula that opens onto the back of the pup's tongue. With this preparation liquid diet can be delivered when the pup is attached to the nipple of its anesthetized mother and suckling, or when it is away from the mother.

The ingestive response the pup displays depends on whether or not it is suckling. When liquid-diet injections were made into the mouths of 5-day old pups suckling at the nipples of their anesthetized mothers, pups rapidly consumed diet and showed the stereotypic ingestive response associated with this form of ingestion. When the identical diet was injected into the mouths of pups which were not suckling; lapping, swallowing, and diet spillage occurred.

Intake regulation also differs for these two modes of ingestion. When 5-day old pups (6 hr food deprived, n=10) were allowed to freely consume diet (delivered in pulses through the intra-oral cannulae, 0.1cc/min) while suckling from their anesthetized mother, they virtually drowned themselves before rejecting the nipple, consuming 100% of the injected diet (11.2% body weight). Similarly treated pups (n=6) fed the same diet away from the mother consumed only a portion of the injected diet and showed no general behavioral response to the diet-deliveries (e.g. on a 1-10 scale with 10 being highest activity, activity count before deliveries start, $\bar{X}$=2.3; during deliveries, $\bar{X}$=2.1). If deprivation was increased to 22 hr, however, the intake of suckling pups declined, while the intake of pups fed away from the nipple increased. Significantly, these pups now also showed a behavioral activation in response to diet-delivery (activity count before deliveries start, $\bar{X}$=3.4, during deliveries, $\bar{X}$=7.1, and pups now vigorously licked the floor).

Thus, the context of whether or not the pup is attached to the nipple appears to determine which of two ingestive responses the pup will emit when liquid diet is delivered intra-orally. Little or no intake regulation occurs when the 5-day old pup consumes diet in the suckling mode. Intake accomplished by elicited lapping and swallowing shows an adult-like increase to prolonged deprivation and suggests an early nutritional control of this precursor to adult ingestive behavior.

499 REDUCED TOLERANCE TO d-AMPHETAMINE-INDUCED ANOREXIA AFTER 6-HYDROXYDOPAMINE INJECTIONS WHICH DEPLETE BRAIN DOPAMINE. Thomas G. Heffner* and Lewis S. Seiden, Department of Pharmacological & Physiol. Sci., U. of Chicago, Chicago, IL 60637

Development of tolerance to the anorexic effect of d-amphetamine was examined in rats given a 6-hydroxydopamine (6-HDA) treatment designed to selectively destroy central dopaminergic neurons. Groups of rats were given i.p. injections of pargyline (50 mg/kg) and desmethylimipramine (25 mg/kg) prior to intraventricular injections of either 200 ug 6-HDA or the vehicle solution (controls). Assays for telencephalic catecholamines performed at the end of these experiments revealed that the pargyline, desmethylimipramine and 6-HDA treatment reduced the levels of dopamine by 75-85% with no change in norepinephrine levels. Beginning two weeks after these treatments, all rats were restricted to 4 hr access to food daily and intake was measured after the first hr. d-Amphetamine was injected i.p. 30 min prior to the start of the access period. In phase I of the experiment, dose-effect functions for d-amphetamine were established with three non-drug days separating each test day. As previously reported,rats given 6-HDA showed resistance to d-amphetamine-induced anorexia (ED_{50}=2 mg/kg) compared to controls (ED_{50}= 0.9 mg/kg). In phase II, all rats were given daily injections of the d-amphetamine dose that reduced food intake by an average of 80% in phase I (1.5 mg/kg for controls, 3.0 mg/kg for rats given 6-HDA). Controls rapidly developed a partial tolerance to d-amphetamine-induced anorexia; food intake rose from 22% of pre-drug levels on day 1 to approximately 55% of pre-drug levels on days 4-21. However, food intake in rats given 6-HDA was only 25% of pre-drug levels after 21 days of d-amphetamine injections. In phase III, dose-effect functions for d-amphetamine were redetermined while giving the same d-amphetamine doses used in phase II on non-test days. Control rats showed a shift to the right in the d-amphetamine dose-effect curve (ED_{50}=1.8 mg/kg) as compared with the dose-effect curve determined in phase I. On the other hand, the dose-effect curves determined in phases I and III for rats given 6-HDA were identical. The failure of rats given 6-HDA to display tolerance to d-amphetamine does not seem to be due to a general inability of such animals to display tolerance to anorexic agents since another group of rats given the same 6-HDA treatment as above showed complete tolerance to the anorexic effects of fenfluramine over the same time course as controls. These data suggest that central dopaminergic neurons are involved in the development of tolerance to the anorexic effects of d-amphetamine. (Supported by PHS MH-11191-12; RCDA PHS MH-10562-11; MH-14274-02, Trng).

500 MUTUAL SUPRA-ADDITIVE FACILITATION OF CONTRALATERAL SELF-STIMULUATION AND STIMULATION-INDUCED FEEDING. Luis Hernandez* and Bartley G. Hoebel, Dept. of Psychol., Princeton University, Princeton, N.J. 08540

In previous experiments we found that lesions of the medial or lateral hypothalamus (LH) affected free feeding and contralateral self-stimulation alike. For example, unilateral electrolytic lesion of the LH decreased temporarily both feeding and contralateral LH self-stimulation, and both recovered in parallel. It is also known that self-stimulation of one side of the hypothalamus facilitates self-stimulation of the other side. We reasoned that because feeding and self-stimulation (SS) are correlated and SS exhibits bilateral facilitation, then electrically elicited feeding should be facilitated by contralateral, suboptimal self-stimulation trains and vice versa, self-stimulation-bound feeding (SBF) trains. Methods: Rats with bilateral monopolar LH electrodes that produced SS and SBF at both electrode placements were used in the experiment. For self-stimulation we used .5 sec trains of 100 Hz,.1 msec pulses. These short trains would not produce SBF. For SBF we used 5 sec trains alternated with 5 sec off. Current intensity was considered suboptimal for SBF when it produced less than five feeding responses in ten presentations, and suboptimal for self-stimulation when it produced less than half of the maximal rate of lever presses during a 20 min test. There were four bilateral interaction tests: a) SS plus auto-SS: each rat pressed a lever for suboptimal SS at one electrode while suboptimal SS coincident trains were also automatically delivered to the other side. b) SS plus SBF: again the rats worked for SS, but this time suboptimal SBF current was delivered continuously for 20 min to the other side. c) SBF plus auto-SS: SBF with suboptimal current was tested while coincident SS trains at 0.5 sec on, 0.5 sec off, were delivered contralaterally. d) SBF plus SBF: again SBF was tested, but with contralateral, coincident SBF trains. Results: SS on one side of the hypothalamus was facilitated significantly by either SS or SBF trains delivered to the other side. The opposite also occurred; SBF on one side was facilitated by either SS or SBF trains on the other side. The important point is that the behavioral response with bilateral stimulation was 4.5 to 7 fold greater than with unilateral stimulation in each of the four cases. This suggests that, similar to facilitation of spinal root stimulation, unilateral hypothalamic stimulation produces facilitation by means of a "subliminal fringe" of excitation in a network common to both sides and common to both SS and SBF. It is concluded that contralateral feeding and reward elements interact synergistically at a common site of convergence.

501 MICROINJECTIONS OF GABA OR BICUCULLINE INTO BRAINSTEM SITES: EFFECTS ON FEEDING. J. Kelly*, G.F. Alheid*, A. Newberg*, and S.P. Grossman. Dept. of Behavioral Sci., Biopsychology Section, Univ. of Chicago, Chicago, Ill. 60637.

Microinjections (100 ng in 1 ul) of bicuculline methiodide into the lateral hypothalamus of rats reliably increased the intake of sweetened milk. Animals with placements in the ventromedial hypothalamus displayed opposite, inhibitory effects of bicuculline on food intake and marked facilitatory effects of microinjections (100 ng in 1 ul) of GABA. Animals with cannula placements in the ventral tegmental area reduced food intake after GABA. Bicuculline injections into this region sharply increased the general level of activity and this, in turn, interfered with drinking. Neither bicuculline nor GABA affected food intake when administered to the substantia nigra. Repeated injections of bicuculline resulted in extreme and persistent hyperactivity accompanied by unidirectional "barrel rolling". GABA injections reversed these effects within a few minutes. The pattern of drug effects suggests that the effects of direct or indirect manipulations of hypothalamic catecholamines on food intake may be mediated, at least in part, by GABAergic mechanisms.

502 EFFECT OF LATERAL HYPOTHALAMIC LESIONS ON INGESTIVE BEHAVIORS IN RHESUS MONKEYS. Joseph W. Kemnitz, Gary W. Kraemer, Richard E. Keesey and Robert W. Goy. Primate Research Center, Univ. of Wisconsin, Madison, Wisconsin 53706.

There is a voluminous literature documenting the effects of lateral hypothalamic (LH) lesions in rats. In this species, LH lesions normally produce transient aphagia and/or anorexia, chronically reduced body weight, and altered responsiveness to a variety of hunger and thirst challenges. However, virtually no data exist regarding the sequelae of LH lesions in primates. With these considerations in mind, this preliminary study is being conducted.

Bilateral radiofrequency lesions were made in the LH of four young adult male rhesus monkeys. The electrodes were acutely placed in the LH utilizing an x-ray ventriculographic procedure. Brief electrical stimulation at the lesion site prior to producing the lesions elicited a small increase in blood glucose ($\bar{X}$= 9 mg%, n=3) measured 15 min. after stimulation.

Following surgery, all animals were hypophagic for at least one week. When animals would refuse to in-quest their usual fare of Purina biscuits, they would eat preferred food such as fresh fruit whenever it was offered. Idiosyncratic behaviors associated with eating (e.g., biscuit rubbing) were not affected by the lesions.

Fluid intake was also reduced for at least several days postlesion. Two animals were drinking normal amounts of tap water within one week following surgery, one animal would reliably drink only flavored water (orange Tang) for several weeks postlesion, and one animal would not drink more than 100 cc/day (i.e., about 10% of normal) of the tap water or flavored water for two weeks postlesion.

Other behavioral and physiological measures are currently being taken, including body weight, fasted levels of blood glucose and several hormones, glucose tolerance, insulin challenge, and catecholamine turnover. Glucose tolerance functions obtained while the animals were sedated with ketamine were not different in intact and lesioned animals 2-5 weeks postlesion.

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503 FRONTAL NEOCORTEX AND FEEDING BEHAVIOR IN RODENTS. Bryan Kolb, A. Nonneman and Ian Q. Whishaw. Dept. Psychology, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

In recent studies we have suggested that the frontal cortex of rodents plays an important role in feeding and drinking. We have extended the examination of this role in 5 different ways: 1) Discrete lesions to subregions of the frontal lobe demonstrated that the ventral lateral frontal (VL) cortex was the focus of feeding deficits in rats, hamsters, and guinea pigs. There was no significant correlation between the amount of cortical tissue removed and length of aphagia unless the VL cortex was removed. The feeding deficits were not due to a general deterioration of motor functions, a failure to initiate behavior, or sensory neglect. However, the animals may be unable to chain together the sequences of motor acts necessary for feeding. 2) Manipulation of body weight affected the length of aphagia. Preoperatively fattening rats to 120% of original weight significantly prolonged the aphagic period whereas dieting the rats to 80% had little effect. Both fattened and dieted rats assumed a chronic postoperative weight level that was independent of preoperative weight level and was 25% of control level. 3) Removal of all of the frontal cortex in neonates (less than 10 days of age) or juveniles (25 days of age) failed to alter food intake, induce motor deficits or chronically lower body weight. Complete decortication at 7 days of age also failed to produce aphagia or motor deficits although the rats had a chronic weight drop of about 35% of control level. 4) Animals with lateral hypothalamic (LH) lesions induced 30 days after VL frontal lesions failed to show aphagia or adipsia whereas rats with prior frontal cortex lesions which spared the VL tissue exhibited the complete "lateral hypothalamic syndrome". This phenomena of recovery after VL lesions could be interpreted in terms of concepts such as denervation supersensitivity. However further work suggests it could be at least partly due to the original drop in body weight. If rats with VL lesions are fattened to control level prior to the LH lesion, they are aphagic. 5) Finally, combination LH and contralateral VL lesions produce aphagia and adipsia similar to that observed after bilateral VL lesions whereas unilateral LH or VL lesions have little effect on feeding.

These results are discussed in terms of cortical-brainstem interactions as well as cortical and subcortical reorganization of function.

504 CHOLECYSTOKININ (CCK) INHIBITS TAIL-PINCH INDUCED EATING IN RATS. Morris A. Lipton, Albert J. Osbahr, III,* Charles B. Nemeroff Gloria D. Jahnke,* Garth Bissette* and Arthur J. Prange, Jr.* Biological Sciences Research Center and the Dept. of Psychiatry, Univ. North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

Cholecystokinin (CCK) is a peptide hormone of intestinal origin which stimulates the secretion of glucagon and insulin. It consists of 33 amino acids, though the C-terminal octapeptide possesses all of the biological activity of the parent compound. When administered IP, CCK induces behavioral satiety in rats and monkeys (Gibbs et al., 1973, Nature 245:323; Smith and Gibbs, 1975, Pharmacol. Biochem. Behav. 3:135). In the present study the effect of the C-terminal octapeptide on tail-pinch-induced ingestive behavior in the rat has been evaluated. The tail-pinch paradigm utilized was that of Antelman and Szechtman (Science 73, 731, 1975). Adult male Sprague Dawley rats (200-250 g) were initially screened with a 2 minute tail-pinch and rats not responding with the characteristic tail-pinch behavior (eating and gnawing) were discarded. On the day of testing rats were randomly divided into 2 groups ($n \geq 6$) and injected IP with CCK (0.1-100 µg/kg) or vehicle (0.9% NaCl, pH 7.5). Five minutes later rats received tail-pinch for 10 minutes (approximately 80 psi) in a stainless steel surgical bowl in the presence of 5 grams of food (45 mg pellets). An experienced observer, unaware of the treatment regimen, measured the food consumed during the 10 minute tail-pinch or quantified chewing of wood chips. Peripherally administered CCK in a dose-dependent manner markedly reduced tail-pinch-induced eating. Results are shown in the Table below.

Treatment (IP)	N	Food (grams) Consumed/10 Minute Tail-pinch (χ±SEM)	p<
Saline	24	2.01 ± 0.94	
CCK 0.1 µg/kg	6	1.81 ± 0.50	ns
CCK 1.0 µg/kg	6	1.19 ± 0.38	<0.05
CCK 10.0 µg/kg	16	0.71 ± 0.18	<0.001
CCK 100.0 µg/kg	6	0.20 ± 0.10	<0.01

The incidence of stereotyped chewing of wood chips observed during tail-pinch was not reduced in CCK-treated rats. Peripherally administered bradykinin (100µg/kg) a peptide approximately the same size as the C-terminal octapeptide of CCK had no effect on tail-pinch induced eating. Lateral ventricular injection (n=13 per group) of CCK-octapeptide (500ng) also reduced tail-pinch-induced eating by approximately 50% ($p<0.05$). This is the first demonstration that CCK exerts behavioral effects after central administration. (Supported by NINCDS NS-05722, HD-03110, MH-22536 and MH-15631).

505 VAGAL MEDIATION OF A VISCERAL DRINKING SYSTEM IN THE RAT. James R. Martin, Paula J. Geiselman, and Donald Novin. Department of Psychology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Following recovery from bilateral subdiaphragmatic vagotomy (vagx), rats showed a lower daily water intake than did laparotomized controls. Daily food intake in the vagx rats, however, did not differ from control levels. In both groups, intraperitoneal injection (1 ml/100 g BW) of 4.5% NaCl resulted in a shorter latency to drink and greater water consumption during a .5-hour testing session than did .9% NaCl. No difference was found between groups following injection of .9% NaCl; however, the vagx rats showed a greater latency and lower intake than did the controls subsequent to injection of 4.5% NaCl. Maintenance of vagx and control rats on a liquid diet did not alter the deficit observed in the vagx group, thus precluding the possibility that the attenuated response of vagx rats to a hypertonic challenge could have been due to excessive gastric retention, which is characteristic of vagx rats fed a solid diet. Using a more selective procedure, vagal fibers joining the liver immediately below the diaphragm were transected; however, this intervention had no effect on drinking elicited by intraperitoneal administration of 4.5% NaCl.

In further experimentation, total vagx and control rats were implanted with either a chronic hepatic-portal or jugular cannula and were infused with buffered Ringer's solution, which included either .9% NaCl or 4.5% NaCl (.5 ml/100 g BW). In comparison to isotonic infusion, latency was shorter and water intake was greater following hypertonic infusion in both vagx and control rats. Isotonic infusion via the two routes in vagx and control rats did not result in any differences in latency or magnitude of drinking. The quantity of water consumed by the vagx and control animals following hypertonic infusion via the two routes did not differ appreciably. However, the latency to drink was shorter subsequent to the administration of the osmotic stimulus via the hepatic-portal route in control animals than in the other groups.

In summary, this series of experiments provided evidence for a vagally-mediated visceral osmosensitive drinking system. The role of this peripheral system will be discussed within the context of the previously established brain osmoreceptor system.

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506 THE EFFECT OF TRIGEMINAL DEAFFERENTATION ON FOOD AND WATER INTAKE IN THE RAT. Maria G. Miller* (SPON: R. M. Wylie). Walter Reed Army Institute of Research, Washington, DC 20021 and Barnard College, Columbia University, New York, NY 10027.

To study the relative roles of peripheral and central factors in controlling food intake I selectively deafferented the oral cavity of rats. A method was developed for individual sectioning of the alveolar, palatine and lingual branches of the trigeminal nerve that convey somatosensory inputs from the mouth. Proprioceptive and motor innervation of the mastication musculature is spared as well as taste carried by the VIIth and IXth cranial nerve. (1) Deafferentation of either the lower part of the oral cavity including the tongue or the upper part led to a reduction in average food intake (mash) lasting for several weeks and to retardation of recovery of the lost body weight. Variability of intake from day to day was increased. (2) After partial deafferentation affecting both the upper and lower part of the mouth the animals failed to eat mash. If offered four diets differing in texture and palatability the animals continued to ingest the preoperatively most preferred semi-liquid cereal and to a lesser degree moist pellets though in insufficient amounts for maintaining their body weight. Water intake was severely depressed or abolished. Intake sufficient to lead to recovery of body weight began only after weeks but then occurred abruptly. (3) Complete deafferentation of both the upper and lower part of the mouth including tongue led to adipsia and aphagia lasting a minimum of five weeks at which time some animals started to recover. These findings establish that peripheral trigeminal deafferentation of the oral cavity significantly impairs the rat's ability to maintain adequate intake.

Behavioral tests and observations demonstrated that the animals were able to bite, chew and swallow. I observed no evidence of general debility or deafferentation neuralgia. However, impairment in coordination of the consummatory act was suggested by a reduction of ingestion rate and inappropriate oral behavior in biting tests and during exploration of food. By increasing the duration of feeding partially deafferented animals compensated for these deficits but not sufficient to maintain normal body weight. Completely deafferented animals failed to show compensation making only few and short attempts to eat and drink. Therefore, it is concluded that in the rat peripheral trigeminal deafferentation also affects the propensity for feeding and drinking.

507 THIRST NEURAL CIRCUITRY: EFFERENT CONNECTIVITY OF THE SUBFORNICAL ORGAN DETERMINED BY AUTORADIOGRAPHY. Richard R. Miselis, Peter J. Hand and Robin Berger*. Animal Biol., Sch. Vet. Med. and Inst. of Neur. Sciences, Univ. of Penn., Phila., PA 19104.

Little is known of the neural connectivity of the subfornical organ(SFO), one of the circumventricular organs of the brain. Its functions have been speculative up until the demonstration of the SFO as a receptor site for angiotensin II in the induction of thirst(Simpson and Routtenberg. Sci. 181: 1172, 1973). A study of the efferent projections of the SFO offers an opportunity to identify neural circuitry involved in thirst and related physiological mechanisms. In addition, identification of the efferent projections may suggest new functions by virtue of the terminal fields found. The autoradiographic technique of tracing labeled amino acids within axons to their synaptic terminations was used to reveal the SFO's efferent projections. 20 nl of a ^{3}H-leucine solution(25 μCi/μl) was slowly infused(25 min) through a 33 gauge beveled cannula(bevel facing caudad) stereotaxically aimed for the mid-dorsal border of the rat SFO. The time to sacrifice and commencement of autoradiographic-histological procedures was 2 days. Control injections were made in adjacent septal nuclei and in the cerebral ventricles. The septum and preoptic, anterior and lateral hypothalamus were examined for sites of terminal transport. The SFO projects to the nucleus medianus(NM), the organum vasculosum lamina terminalis(OVLT) and the supraoptic nucleus(SON) bilaterally. Injections that involve the SFO unilaterally showed greater grain densities over the ipsilateral SON. The increased grain densities appeared to have occurred via axonal transport and not via vascular or ventricular routes since neighboring vascular endothelium and ependyma did not show increased grain densities. Projections to septal nuclei could not be demonstrated nor excluded. Control injections into septal nuclei did not label the above sites. Ventricular injections produced increased grain densities in the OVLT and medial preoptic region but was mostly confined to the ependyma. These data suggest the involvement of the NM and OVLT in neural mechanisms of thirst. They also suggest a modulatory role of the SFO on the SON. (Supported by NS-06716)

508 Degenerated Neuroanatomical Pathways Associated With Aphagia And Adipsia Following Lateral Hypothalamic Lesions. Elliott Mufson* and Walter Riss, Program in Biological Psychology, and Department of Anatomy and Cell Biology, Downstate Medical Center, Brooklyn, New York, 11203.

The importance of the lateral hypothalamus(LH) in the regulation of ingestive behavior is well established. Bilateral electrolytic damage of the LH at the level of the ventromedial hypothalamic nucleus (VMH) disrupts feeding (aphagia) and drinking (adipsia) (Anand and Brobeck, 1951;Teitelbaum and Epstein, 1962).

Several authors have stressed that ingestive behavior depends on the interaction of the lateral hypothalamus with other levels of the neuraxis. This concept is supported by data demonstrating that feeding and drinking are disrupted by damage to extra hypothalamic areas such as the amygdala, the central gray matter, and the reticular formation. The present investigation is a correlative behavioral and anatomical analysis of the neural substrate underlying aphagia and adipsia following LH damage. Specific attention is directed towards the anatomical interfaces between the LH and other areas known to be involved in the acts of feeding and drinking. Adult rats of both sexes were used. The strategy was to place bilateral electrolytic lesions varying in size in the LH at the level of the VMH and correlate post-operative ingestive deficits with the pattern of degeneration revealed by the Fink-Heimer I or II procedure (1967).

Daily food and water consumption were recorded both pre and post-operatively. Lesion parameters were 1 ma for 3,7 and 10 sec. Animals were allowed to survive for 1-14 post-operative days. Only the 7 and 10 sec. groups were aphagic and adipsic. Since the pattern of degeneration was qualitatively similar for all lesion groups the data described is from the smallest effective LH lesion (i.e., the 7 sec. group).

Degeneration was traced from the lesion to the central amygdaloid nucleus, lateral preoptic area, medial and lateral septum, nucleus accumbens and lateral habenular nucleus. Descending degeneration coursed to the tectum, midbrain central gray, substantia nigra pars compacta and reticulata,mesencephalic tegmentum and midbrain raphe nuclei. Degenerated fibers entered the medial pontine gray at the level of the mesencephalic-metencephalic junction. Further caudally, degenerated axons were observed in the dorsal and ventral parabrachial region and motor and mesencephalic nuclei of the trigeminal nerve. All lesions damaged the medial edge of the internal capsule producing degeneration in the crus cerebri-pyramidal fiber system. In certain brains fibers were seen in the VMH nucleus. Many of the structures which receive a projection from the LH complex are also involved in the regulation or expression of ingestive behaviors, judging by existing evidence.

509 ALCOHOL CONSUMPTION FOLLOWING HIPPOCAMPAL LESIONS IN MICE. J. N. Pasley* and E. W. Powell. Dept. Physiol. and Anat., UAMSC, Little Rock, AR 72201.

Thirty mature wild-derived male house mice were selected for this study. Ten animals sustained bilateral lesions to the posterior (hilar) segment of the hippocampus. Ten other animals sustained bilateral lesions to the amygdala, or ventral hippocampus. After a 7 day surgical recovery period, the mice, including an additional ten unoperated animals, were given access to both water and a 5% solution of ethyl alcohol in a free-choice situation. Ethanol consumption in animals which sustained bilateral lesions of the posterior hippocampal area averaged from 34% to 69% of their total fluid intake over the two week test period. The control group which sustained lesions in parahippocampal structures did not exhibit this differential and their proportion of alcohol intake to total consumption was nil (0-4%). The ten unoperated mice also rejected the 5% ethanol solution. Thus, it may be possible to attribute a degree of voluntary alcohol consumption in mice to select limbic system balances.

510 ARE THE CENTRAL EFFECTS OF ANGIOTENSIN DUE TO PERIPHERAL ANGIOTENSIN II CROSSING THE BLOOD BRAIN BARRIER? M. Ian Phillips, Pushpa Deshmukh & William Larsen. Dept. Physiology, Dept. Anatomy University of Iowa, Iowa City, Iowa, 52242.

Angiotensin II (AII) injected into the brain ventricles (IVT) causes a blood pressure increase, drinking and vasopressin release to ensue. AII injected i.v. however, does not elicit vasopressin release and there are subtle differences in the i.v. and IVT effects of the peptide on BP and drinking. Autoradiographic studies indicated that the AII i.v. crosses the blood brain barrier (BBB) and gains access to the ventricles. In these studies the doses used were extremely high (2 μg+).

To investigate if AII at high doses was causing a change in the BBB we used 68 male, adult rats injected with horseradish peroxidase (HRP), 1-50mg, Sigma type VI, alone or with AII, i.v. and IVT. Blood pressure was recorded. Ten minutes after injection, the rats were perfused with glutaraldehyde and the brains sectioned for reaction and light and electron microscopy.

HRP injected i.v. alone lowered BP. The peroxidase reaction product was seen in the blood vessels and pericytes, and in the tissue of the circumventricular organs (CVO) and choroid plexus, as Brightman, Cancilla and others have shown. With 2μg AII + HRP i.v. there was a rise in BP (70mmHg) and the reaction product was observed outside the blood vessels in additional areas of the striatum and hypothalamus. This increased permeability of the BBB could allow AII to reach ventricular receptor sites from blood.

HRP injected IVT penetrated the ciliated ependyma wall but not the CVOs. With AII + HRP IVT, penetration was deeper into the brain parenchyma, but not into the CVOs.

Freeze fracture of the rat ventricular wall showed gap junctions at the apical tips of ciliated ependyma. It is possible that AII causes greater penetration by altering the state of the gap junctions. In the CVOs more tight junctions are found and we report here the finding of a type of tight junction in the subfornical organ. Tight junctions would resist entry of AII from the CSF.

Although the evidence with HRP (MW43000) is indirect, it suggests that a smaller molecule such as AII (MW1000) injected i.v. could cross the BBB if blood pressure is substantially raised. With lower doses, however, peripheral AII would not reach the same sites as AII injected centrally. Thus the central effects of AII IVT injections may be due to stimulation of receptors which are not reached by low levels of peripheral AII.

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511 METABOLIC CORRELATES OF DISSOCIATED FEEDING RESPONSES INDUCED BY HEPATIC-PORTAL AND DUODENAL INFUSIONS (HPI and DI) OF SMALLER AND LARGER LOADS (SL and LL) OF ISOTONIC GLUCOSE. EFFECTS OF SOMATOSTATIN (SRIF) AND CYCLOHEXIMIDE.
M. Rezek, V. Havlicek, and H. Friesen, Dept. of Physiology, U. of Manitoba, Winnipeg, Manitoba, Canada.

Hepatic-portal infusions of SL (10 ml) and LL (30 ml) of glucose, which had previously been shown to have little or no effect on food intake in rabbits (Rezek et al., Am. J. Physiol. 1975), produced an immediate, sharp rise in glycemia which peaked briefly at the end of the respective infusions (10 min and 30 min) and then declined rapidly. Maximal increases in glycemia for SL and LL were 22% and 53% resp. Serum insulin responses were also rapid, rising quickly to their respective peaks at the end of the infusions (SL at 10 min = 17 μU increase; LL at 30 min = 30 μU increase). The post-peak decline of insulin levels was rapid and reached steady state at 30 min (SL) and 60 min (LL). In contrast, the responses of blood glucose and insulin to duodenal infusions of SL and LL, which in chronic experiments produced satiety (SL) or paradoxically stimulated food intake (LL), were considerably different. The glucose response to SL was immediate: it peaked briefly (23% increase) at the end of the infusion (10 min) and then declined more slowly than it had after portal SL and reached steady state at 60 min. On the other hand, the glucose response to LL, although immediate and fast rising initially, required 60 min to peak (> 80% increase); the subsequent decline was slow so that at the end of the test period (3 hrs) glycemia was still considerably elevated. The insulin response to SL was immediate and rapid although slower than after HPI (max. increase 21 μU at 30 min); however, the subsequent decline was rapid transiently and at 60 min it had practically reached steady state. Thereafter the decline toward the preinfusion baseline level required an additional two hours. In marked contrast, the insulin response to LL, although initially immediate and rapid approached its maximum gradually and at a considerably higher level (max. increase 55 μU at 60 min). Its decline was rather slow and as a result the insulin level in the end of the 3rd hour was still markedly elevated (> 30 μU). This prolonged, intesified insulin response represents the main difference when compared with responses to DI of SL as well as to HPI of SL or LL and correlates with the dissociation of the corresponding feeding responses. This marked and prolonged insulin increase as well as the paradoxical overeating induced by DI of LL are both reduced or eliminated by pretreatment with SRIF and cycloheximide. These data suggest that the expression of paradoxical overeating may be dependent on the prolonged release of insulin from both the acute as well as chronic insulin pools. Supported by MRC Canada.

512 A PHARMACOLOGICAL ANALYSIS OF CHOLINERGIC AND PEPTIDERGIC DRINKING AT THE SUBFORNICAL ORGAN. John B. Simpson and Michael L. Mangiapane* (SPON: Nancy J. Kenney), Department of Psychology, University of Washington, Seattle, WA 98195

The subfornical organ (SFO) has been identified as a particularly sensitive site of drinking elicited by intracranial cholinomimetics (Simpson and Routtenberg, Brain Research, 79, 1974) and by the hormone, angiotensin II (Simpson and Routtenberg, Science, 181, 1973). An important question is that of the relationship between the receptors for the two dipsogenic substances. Further, the identification of the SFO as a structure of unique sensitivity for both forms of elicited drinking makes it amenable to pharmacological analysis of a degree of specificity which is not possible at alternative loci, such as the cerebral ventricles. Carbachol (CBC) drinking at the SFO was characterized by pretreatment at the structure with various cholinergic antagonists (in 0.5 ul) prior to CBC (100 ng/0.5 ul). The nicotinic antagonists hexamethonium and decamethonium did not affect CBC drinking at molal ratios of antagonist:agonist of 1:1, 10:1, or 100:1. Drinking was also unaffected by equimolal pretreatment with d-tubocurarine prior to CBC. Alternatively, the muscarinic antagonist atropine significantly reduced or eliminated drinking to CBC at molal ratios of antagonist:agonist of 1:1, 1:10, and 1:100. Hence, cholinergic thirst at the SFO is mediated by muscarinic cholinergic receptors. The drinking induced by angiotensin II (A II; 10 ng/0.5 ul) at the SFO was significantly reduced by the A II antagonist, Saralasin Acetate, at molal ratios of antagonist:agonist of 1:1 and 10:1. Hence, A II drinking at the SFO is mediated by A II specific receptors.

The interaction between CBC and A II drinking at the SFO was evaluated by using demonstrated antagonists for each dipsogen in competition with the alternative dipsogen. It was found that atropine did not significantly antagonize A II drinking, even at molal ratios of antagonist:hormone of 10:1, 100:1, or 1,000:1 (doses which antagonized CBC drinking). Likewise, Saralasin did not antagonize CBC drinking at the SFO (at doses of antagonist which completely blocked A II drinking). It is apparent, therefore, that drinking induced by cholinomimetics and by A II at the SFO may employ activation of parallel, nondependent receptors. The significance and nature of this apparent pharmacological independence of thirsts at the SFO remains unclear.

513 SHORT AND LONG TERM EFFECTS OF PARA-CHLOROAMPHETAMINE ON CONSUMMATORY BEHAVIORS. J.M. Stein*, M.J. Wayner, K.M. Kantak* and R.C. Cook*. (SPON: C.S. Weiss). Brain Res. Lab., Syracuse Univ., Syracuse, NY 13210.

The possible role of serotonin in eating and drinking was investigated using a single administration of the serotonin neurotoxin para-chloroamphetamine (PCA). Female hooded rats were housed individually and had free access to food and water. Following a baseline period, 0.9% saline or 1.0,2.0, 5.0 or 10.0 mg/kg of PCA was injected intraperitoneally. Twenty-four hr food intakes were decreased 40-60 % of baseline levels on the day immediately following administration of all PCA doses. Food intakes returned to baseline levels within 48 hr of PCA administration and remained stable for the next 30 days. Twenty-four hr water intakes were decreased 50-60 % of baseline levels on the day immediately following administration of all PCA doses. Significant increases in water intakes, 110-150 % of baseline, were observed following the initial decreases. No further changes in water intakes were recorded during the next 30 days. Additional data will be presented in which the responsiveness of PCA injected animals to salt arousal of drinking and insulin induced eating was investigated. These results will be discussed in terms of the short term changes and lack of long term changes in food and water consumption following PCA.

514 DEVELOPMENTAL PLASTICITY IN RETINO-HYPOTHALAMIC CONNECTIONS AND THE ENTRAINMENT OF CIRCADIAN RHYTHMS. F. K. Stephan* and A. A. Nunez* (SPON: A. Wesley). Dept. Psy., Florida State Univ., Tallahassee, Fl 32306.

The role of retino-hypothalamic pathways in the re-entrainment of drinking rhythms after a 12-hr phase shift in the light-dark cycle was investigated by comparing the rate of re-entrainment of the following groups of rats: (1) unilaterally enucleated as adults (AE), (2) unilaterally enucleated within 24 hr of birth (NE), (3) intact controls (C). As previously reported, AE rats required more days to invert drinking rhythms than controls. On the other hand, NE rats were not significantly different from controls. Previous anatomical observations indicate that neonatal enucleation results in a nearly equal distribution of retinal input to both suprachiasmatic nuclei. Therefore, the results are consistent with the hypothesis that the unequal distribution of retino-hypothalamic fibers in AE rats interferes with the entrainment process. Compared to controls, AE and NE rats showed a smaller reduction in water intake during a 24 hr exposure to constant light. This response, believed to be mediated by the inferior accessory optic tract, was not affected by age of enucleation.

515 THE RENIN-ANGIOTENSIN SYSTEM AND THIRST: A RE-EVALUATION. Edward M. Stricker. Depts. Psychol. & Life Sci., Univ. Pittsburgh, Pittsburgh, PA 15260.

Ligation of the inferior vena cava, and administration of isoproterenol, have been shown to stimulate renin secretion and to augment water intake in rats. Both effects are abolished by bilateral nephrectomy. These results have been used to support the popular hypothesis that the renin-angiotensin system is largely responsible for the mediation of thirst following the two treatments. However, recent experiments suggest that the plasma renin activities produced by caval ligation or isoproterenol treatments do not account for more than 20 percent of the observed drinking behavior. Direct measurements of arterial blood pressure further indicate that nephrectomized rats go into hypotensive shock after either treatment. Drinking can be elicited in these hypotensive animals by systemic injection of hypertonic NaCl solution, renin, or Pitressin, or by intracranial injection of angiotensin, but in each case a rapid increase in blood pressure also is observed. Thus, it would appear that nephrectomy reduces water intake in these animals by undermining their general capacity to behave, rather than by removing a specific dipsogenic stimulus. (Indeed, it is difficult to explain why the removal of the renin-angiotensin system should abolish thirst after caval ligation or isoproterenol treatments, since both treatments would still activate vascular baroreceptors that have been assumed to stimulate thirst during hypovolemia.) These and other results suggest that the renin-angiotensin system contributes little to the direct mediation of drinking that is elicited in rats by caval ligation or isoproterenol.

516 THE ROLE OF THE HYPOTHALAMIC SUPRACHIASMATIC NUCLEUS AS A CIRCADIAN OSCILLATOR IN RAT. Anthony N. van den Pol. Dept. Psychol. Yale Univ.,New Haven, Ct. 06520.

Several previous studies (e.g. Moore & Eichler Brain Res. 42: 201, 1972;Stephan & Zucker PNAS 69:1583,1972) have demonstrated convincingly that the area of the suprachiasmatic nucleus (SCN) is involved in the maintenance of circadian rhythms, but these studies have not ruled out the possibility that fiber systems adjacent to SCN or anterior hypothalamic tissue near the nucleus are also involved in the control of these rhythms. The present experiment evaluated such a possibility using minute radiofrequency (RF) lesions. Lesions about 25 times smaller than those used in earlier experiments were made in and around SCN in 76 male Fischer rats. Other animals received knife cuts (n=14) placed in the SCN area or served as sham-operate (n=15) or blinded (n=10) controls. Drinking and feeding were monitored electronically over a seven week period.

Neural damage restricted to SCN with little or no extra-SCN insult was sufficient to eliminate circadian rhythms of feeding and drinking ($p<.01$). Lesions immediately posterior to SCN had a similar effect, presumably by interupting axonal projections from SCN(Krieg, JCN 55:19,1932). Damage restrictedto the supraoptic commissures, optic chiasm, tractus infundibularis, or the hypothalamic area anterior or lateral to SCN had little influence on circadian rhythms.

As an independent check on the direct lesion analysis, a computer-assisted three-dimensional reconstruction of each rat's RF lesion was made, and the numerical description (autocorrelation) of each rat's postoperative circadian rhythm was correlated with its lesion description. All rats with RF lesions were used. This computer procedure confirmed that damage of the SCN caused a severe impairment of circadian rhythms. A positive correlation ($r=.68$)between the anatomical loci involved in feeding and drinking rhythms was found. Drinking rhythms seemed more resistant to perturbation by SCN lesions than did feeding rhythms. The anatomical area subserving drinking rhythms seemed slightly smaller than that for feeding rhythms when the same autocorrelation criterion was used.

Rats with partial (greater than 10%, less than 85%) SCN damage showed some decrease in circadian rhythm as judged by autocorrelation analysis. Ten rats with more than 50% SCN destroyed showed significant ($p<.05$) eight hour feeding or drinking rhythms; no other rats showed this infradian rhythm.

An additional 107 rats were used to examine SCN with Nissl, Golgi, and ultrastructural methods. The anatomical integrity of SCN in comparison to the surrounding hypothalamus will be discussed. (Supported by PHS AM 11551; ACS IN 31-0-5).

517 DIETARY SELF-SELECTION FOLLOWING DESTRUCTION OF THE LATERAL HYPOTHALAMUS. Dennis A. VanderWeele and Paula J. Geiselman. Depts. of Psychology, Occidental College, Los Angeles, CA 90041, and UCLA, Los Angeles, CA 90024.

Animals sustaining subdiaphragmatic vagotomies have been shown to decrease daily intake of sucrose and increase fat and protein intake compared to preoperative levels (Fox, Kipp, and VanderWeele, Am. J. Physiol. 231:1790-1793, 1976). An afferent vagal-hypothalamic circuit has been postulated to explain the decreased sucrose intake in vagotomized animals (Fox et al., 1976). The present experiments were conducted to assess the effects of destruction of the lateral hypothalamus (LH) on dietary self-selection.

Subsequent to LH damage, rats were evaluated for intake of fat and sucrose while maintained on a protein-Alphacel diet. A significant inverse correlation was found between amount of sucrose consumed and severity of LH syndrome. Comparable analyses for fat intake and daily caloric intake yielded non-significant effects. Severity of LH syndrome was assessed on the basis of final body weight (the lower, the more severe the syndrome) and number of days aphagic (the more days, the more severe the syndrome). In the second study, effects of LH destruction were investigated by comparing preoperative and postoperative consumption of sucrose, casein hydrolysate, and olive oil. LH animals were allowed to recover to Stage II (would ingest wet cookies) at which time measurement of macronutrient intake was resumed. Results indicated that, while there was a tendency for fat intake to decrease, neither protein nor caloric intake was systematically affected by LH destruction. Sucrose ingestion, however, was again found to be inversely correlated with the severity of the LH syndrome. In comparison to preoperative intake, animals that were characterized by a less severe LH syndrome were found to increase sucrose consumption; while sucrose intake in animals with a more severe LH syndrome did not differ from preoperative levels.

In many instances, the vagus and the LH have been found to subserve similar functions. Contrary to prediction, however, the effects of the vagus and the LH on daily sucrose intake are not the same. Results will be further discussed in relation to size and histology of LH destruction.

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518 INHIBITION OF SUCKLING IN THE WEANING AGE RAT BY A DEVELOPING NEUROTRANSMITTER SYSTEM. Christina L. Williams*, Bruce Nock*, and W. G. Hall (SPON: F. S. Kraly). Institute of Animal Behavior, Rutgers Univ., Newark, N.J., and North Carolina Dept. of Mental Health, Raleigh, N.C.

Previous studies have demonstrated the emergence of an inhibitory control of suckling behavior in the infant rat between the 10th and 15th day of life. Prior to this age, nutritional state is not a factor controlling suckling, and all pups will attach and suckle from the nipples of their anesthetized mother, whether deprived or nondeprived. After 15 days of age, only deprived pups will suckle. Since biochemical and behavioral evidence suggest a maturation of serotonergic neural function at this time, we treated 20-day old rat pups with methysergide (20 mg/kg), a putative serotonin (5-HT) receptor antagonist, and immediately observed their suckling behavior with their anesthetized mother for 1 hr.

Methysergide treatment produced a dramatic increase in the suckling of nondeprived pups, compared to saline injected controls (methysergide 37.5 ± 5.6 mins, saline 17.5 ± 8.0 mins, $p<.05$, $n=8$). In addition, methysergide-treated pups, like younger pups, tended to suckle from a single nipple while deprived controls shifted from nipple to nipple.

In order to further assess serotonergic involvement in the mediation of suckling, we treated 20-day old, deprived pups with the putative 5-HT receptor agonist, quipazine (10 mg/kg), alone or with a methysergide (20 mg/kg) pretreatment and observed their suckling behavior 30 mins following quipazine treatment. Quipazine alone, clearly inhibited nipple attachment in deprived 20-day old pups. Pretreatment with methysergide 15 mins prior to quipazine, blocked the inhibitory effects of quipazine on suckling behavior (quipazine 7.5 ± 6.1 mins, methysergide/quipazine 55.6 ± 2.3 mins, $p<.01$, $n=8$).

We have evidence that the reinstatement of suckling which we report, is not simply the result of making the nondeprived pups hungry. Methysergide reliably stimulates suckling in 30-day old pups, but at this age, even 24 hrs of food deprivation fails to activate suckling. Also, given the choice between non-nutritive suckling or food pellets, methysergide-treated pups prefer to suckle while deprived controls prefer to eat food pellets.

In young pups, suckling has the appearance of a fixed reflex behavior. However, in the brief preweaning period, this relatively simple behavior sequentially develops internal controls. The pharmacological stimulation and inhibition of suckling in 20-day old rat pups which we report, suggest that in part, the development of these controls may be related to the emergence of an inhibitory serotonergic system.

INVERTEBRATE NEUROBIOLOGY

519 TWO TYPES OF NEUROMUSCULAR JUNCTIONS IN COCKROACH MUSCLE FIBERS. S. Aizu* (SPON: A. Hess). Dept. Anat., Rutgers Med. Sch., CMDNJ, Piscataway, N.J. 08854.

Different muscles of the coxa of the cockroach, Periplaneta americana, are known to be physiologically fast or slow and to be innervated by fast, slow or inhibitory nerve fibers. Previous investigations of these diverse muscle fibers have thus far failed to find any striking morphological differences in the nerve endings on these muscles. Muscles numbered 177A, 177B, 177C, 177E (e and e'), and 182 have been studied in the electron microscope. Others have shown physiologically that muscles 177A and C receive only fast nerve fibers. The nerve terminals on the fibers of these muscles contain many spherical clear synaptic vesicles and contact the muscle fiber for only a short distance. On the other hand, muscles 177B and 182 are innervated physiologically by three kinds of nerve fibers: fast, slow and inhibitory. In these muscles, two types of neuromuscular junctions are observed. One type is the same as the above mentioned short-contact terminal. The nerve terminal of the other type contains many clear polymorphic synaptic vesicles, and the membranes of nerve terminal and muscle fiber are apposed for a relatively long distance. Muscle 177e receives a dual innervation of slow and inhibitory fibers and both types of neuromuscular junction occur. From the above study, it appears that excitatory terminals of both fast and slow nerve fibers are of the short-contact type, while the inhibitory terminal is of long-contact type. An exception to this conclusion is muscle 177e' which is innervated by fast and slow fibers only (no inhibitory fibers), but in which, nevertheless, both types of terminals have been found. However, it is suggested that muscle 177e' is indeed innervated by inhibitory nerve fibers, which have not yet been found electrophysiologically. Further study of the internal structure and histochemical enzyme patterns of these insect muscle fibers and the nerve terminals on them is under way to attempt to correlate morphologically types of muscle fiber with the type of innervation.

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520 INTERACTION OF CHEMOSENSORY, VISUAL AND STATOCYST PATHWAYS IN *HERMISSENDA CRASSICORNIS*. Tadashi Akaike*, Daniel L. Alkon* and June Harrigan* (SPON: J. Atema) Sec. on Neur. Systems, Lab. of Biophysics, NINCDS, NIH, MBL, Woods Hole, MA 02543.

Synaptic interaction between the visual and statocyst pathways of the nudibranch mollusk *Hermissenda crassicornis* has been recently defined with simultaneous intracellular recordings from neuron pairs during physiologic and electrical stimulation (Alkon 1973, 1975, 1976). To investigate the interaction of chemosensation with sensory perception mediated by the visual and vestibular pathways, intracellular neuronal responses were recorded while stimulating the *Hermissenda* tentacles with palpable food substances, extracts thereof, or amino acids (natural stimulation). Intracellular responses from the same neurons were also recorded during electrical stimulation of the tentacular nerve. Neurons investigated included those with specific loci on the cerebropleural ganglia, photoreceptors within the eyes, optic ganglion cells and statocyst hair cells. Many small neurons depolarized when the tentacle was perfused with squid extract, egg yolk solution or amino acids (glycine, L-proline, D, L-methionine with threshold concentration of 10^{-8} -10^{-10}M). These same cerebropleural neurons depolarized with a fixed latency (5-10 msec) in response to electrical stimulation of the tentacular nerve. Other cerebropleural neurons hyperpolarized with electrical and natural stimulation of the tentacular pathway. The five photoreceptors of each eye hyperpolarized in response to electrical and natural tentacle stimulation. Type A were much less responsive than Type B photoreceptors (e.g. with electrical stimuli Type A hyperpolarized 2-5 mV vs 5-10 mV for Type B). This hyperpolarization of the photoreceptor was associated with a 30-40% increase in membrane conductance as measured by simultaneous impale of single photoreceptors with two microelectrodes. Since the conductance changes were measured within a linear portion of the photoreceptors' current-voltage relation, they cannot be attributed to rectification of the photoreceptor membrane. The hyperpolarization was reversed by hyperpolarizing the photoreceptor, was reversibly eliminated by perfusion with 20 mM $CoCl_2$, and fatigued with repetitive stimulation at 0.2 Hz. Tonic inhibitory potentials in statocyst hair cells were eliminated by natural and electrical tentacle stimuli. These IPSP's most likely arise from ipsilateral optic ganglion cells which were also inhibited by natural and electrical stimulation. Such intersensory interaction may provide a neural basis for a behavioral hierarchy in *Hermissenda* and related species (cf. Davis, 1973). For *Hermissenda*, a positive chemotaxis as well as a positive phototaxis were substantially suppressed when a choice between food substances or a light source was possible (N = 30, $p<.01$).

521 PROLONGED INHIBITION AND EXCITATION IN THE *HERMISSENDA* EYE. Daniel L. Alkon*, and Yoram Grossman* (SPON: T. MacNichol). Sec. on Neur. Systms., LB, NIH, MBL, Woods Hole, MA 02543.

Within each eye of the nudibranch mollusk *Hermissenda crassicornis* there are two Type A and three Type B photoreceptors, distinguished by numerous morphologic and electrophysiologic criteria (Alkon 1973, 1976). Since the somata are only passively invaded by action potentials arising (~80μ away) in the distal axon, the generator response can be studied in the absence of impulses and synaptic interactions (which occur at the terminal branches) by cutting the distal axon (Cut N). Intracellular records from all five photoreceptors (Cut N) after at least fifteen minutes dark adaptation, showed a depolarization for 4-6 minutes after a 30-sec. light step (10^3 ergs/cm^2 -sec). This long-lasting depolarization (LLE), which increased with longer and brighter light steps, was associated with a two to three-fold decrease in membrane conductance although membrane conductance was substantially increased throughout the depolarizing response during the light stimulus. LLE could be eliminated by hyperpolarizing the photoreceptors during the light. Injection of large depolarizing current steps was associated with an increased membrane conductance and was not followed by LLE. Cutting the axon distal to the impulse initiation zone removed all synaptic interactions, but did not alter LLE for either Type A or Type B photoreceptors. When completely intact, the lateral Type A photoreceptor unlike its neighbor, the medial Type A, gave a light response which was followed by almost no LLE and usually hyperpolarized. This long-lasting inhibitory undershoot (LLI) closely paralleled the LLE of simultaneously penetrated Type B and medial Type A photoreceptors. Unitary IPSP's within the LLI followed, one-for-one, impulses of simultaneously penetrated Type B cells. The LLI was eliminated by synaptic blockade with 20 mM $CoCl_2$ or eserine (10^{-3}M). It was associated with small decreases and in some cases slight increases of membrane conductance. This suggested that an increased conductance at the synaptic endings during the LLI added to and mostly cancelled a decreased conductance at the lateral Type A soma. Additional observations (including reduction of LLI by ipsilateral statocyst removal) indicated that the increased firing of Type B cells during their LLE inhibit the lateral Type A by both monosynaptic and polysynaptic (via statocyst hair cells) inhibition. When excitation of these hair cells by rotation was paired with a light step, the LLE of Type B cells was substantially enhanced and the number of unitary IPSP's during the LLI of the lateral Type A was correspondly increased. This enhancement of LLE and LLI can be explained by changes of known synaptic interactions and may help explain previously observed neural and behavioral changes produced by associative training of intact *Hermissenda* (Alkon 1975).

522 ACTIVATION OF A STOMATOGASTRIC MOTOR PATTERN GENERATOR BY DOPAMINE AND L-DOPA. William W. Anderson* and David L. Barker, Bio. Dept., U. of Oregon, Eugene, OR 97403.

Biochemical and histochemical data indicate that neurons in the spiny lobster stomatogastric ganglion (SGG) are innervated by dopaminergic fibers originating from the paired commissural ganglia (CG) (Barker and Hooper, Neurosci Abst 1:395, Kushner and Maynard, Brain Res, in press). When the nerve connecting the CG and SGG is cut, the pyloric motor output from the SGG decreases 2-4 fold in frequency and only 3 of the 13 pyloric motorneurons remain active (Russell, J Exp Biol, in press). To assess the role of dopamine (DA) in the activation of pyloric motor output, the isolated SGG was bathed in saline containing DA or its precursor L-DOPA. L-DOPA was used with the aim of inducing excess synthesis and spontaneous release of DA at the proper synaptic sites. We found rapid conversion of ^{3}H-DOPA to ^{3}H-DA; ^{3}H-norepinephrine synthesis was not detected.

Both DA (50-200 μM for 30 min) and L-DOPA (20-200 μM for 2 hrs) induced gradual activation of three pyloric motorneuron types (2-PD, LP, 8-PY) beginning after 2-5 min in DA and 9-30 min in L-DOPA. Although the degree of activation was variable, depending in part on the time between CG removal and stimulation, the pyloric output of strongly activated preparations was nearly identical whether induced by DA or L-DOPA, and was similar in many respects to the intact CG-SGG preparation. The previously silent LP and PY neurons began rhythmic firing in the normal PD-LP-PY sequence, and cycle frequency of the PD bursting pacemaker neurons increased to CG-SGG levels. However, IC activation was rare, VD activity often decreased, and the number of PD spikes/burst was less than in the CG-SGG preparation. During washout, DA activated preparations returned to control levels within 30 min, while L-DOPA activated preparations continued to increase their activity for 2-3 hrs and required at least 5 hrs to reach control levels. The differences in time course indicate that L-DOPA does not act by directly stimulating receptors, but through conversion to, and release as, DA.

Our results suggest that dopaminergic input could be a major component of CG activation of the pyloric motor pattern generator, and that tonic release of a particular neurotransmitter may activate the coordinated rhythmic output of several neurons in a pattern generator. (Supported by NIH grants NS-10614 and GM 00336.)

523 PHOTORESPONSIVE NEURON ON THE VENTRAL SURFACE OF THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. Michael C. Andresen* and Arthur M. Brown (SPON: D. C. Eaton). Dept. of Physiol. & Biophysics, Univ. of Texas Medical Branch, Galveston, Texas 77550.

A number of neurons in the central nervous system of Aplysia respond directly to illumination. Most of these neurons require fairly high light intensities. This report describes a ventral photoresponsive neuron (VPN) whose threshold light requirement is 1000x's lower than for the photoresponse of R_2 and is comparable to the threshold for the Aplysia eye. When VPN is voltage clamped near the resting potential, brief (100 msec) illumination increases the membrane conductance and produces an outward light-induced current (I_L) lasting about one minute. The reversal potential of I_L and its shift with changes in external potassium are consistent with the Nernst equation for potassium. The I-V curves for I_L are nonlinear and agree with the constant field equation. The recovery phase of the I_L response consists of two exponential components with time constants of 12 and 50 seconds. When illuminated, the pigmented lipochondria in the cytoplasm of VPN undergo morphological changes similar to those found in R_2 and associated with a release of calcium. The similarities of the photoresponses of VPN and R_2 suggest that an identical mechanism is responsible for the phototransductive properties of these neurons.

524 BIOSYNTHESIS OF NEURO-DEPRESSING HORMONE (NDH) IN THE NERVOUS SYSTEM OF THE CRAYFISH. Hugo Aréchiga, Casimiro Cabrera-Peralta, Alberto Huberman* and Irene González* Dept. Physiol. Ctr. Adv. Stud., IPN, México, and Dept. Biochem. Inst. Nutr. Dis., México, D.F.

The nervous system of the crayfish contains a neuro-hormone of peptidic nature, which depresses neuronal excitability. The greatest amount of this substance is contained in the sinus gland, a neuro-haemal organ in the eyestalk, wherefrom it is released (Aréchiga, Huberman and Martínez-Palomo, Brain Res., 1977 In Press). The purpose of this work is to locate the site(s) of synthesis of this hormone. In a first stage, 200 adult specimens of Procambarus bouvieri (Ortmann) of either sex were subjected to eyestalk ablation, and the content of NDH in blood and in the remaining of the nervous system was determined at 24-hour intervals. NDH was separated from crude extracts, passing the material successively, though Sepahdex G-25 and G-10 columns, and identifying by bioassay, the active fraction thus separated from other peptides. The activity was meassured as the decrement in spontaneous firing rate in a set of identified motoneurons in the 3d abdominal ganglion. NDH content gradually diminished both in the nervous system and in blood after eyestalk excision until nearly disappearing in 4 days. Yet, some recovery was apparent at the end of one week. A similar time course was found in a second group of animals in which the eyestalks were ligated and left in place. In order to test the possibility of synthesis in vitro, the eyestalk ganglia were excised, and incubated in culture medium (Eagle's MEM) at 20°C for variable periods of time. Two types of preparations were used; a) intact ganglia, devoid of sinus gland, and b) homogeneized ganglia, from which NDH originally contained was removed by dyalisis. In this latter preparation, incubation was ended by heating at 75°C for 3 min followed by centrifugation for 1 min and the supernatant was taken and passed to Sephadex. In both, NDH was present after a few hours, and in the intact ganglia, NDH activity was also present in the culture medium. These results indicate that the neurosecretory apparatus of the eyestalk synthesize NDH, and that normally it is the main source of this hormone. That it is not the sole source is suggested by the recovery of NDH activity in eyestalkless animals.

525 SHORT AND LONG-TERM ERG CHANGES IN BRAINLESS CRAYFISH. Baltazar Barrera-Mera and B. Fuentes-Pardo. Depto. de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México 20, D.F. MEXICO

Cerebral ganglion lesion studies (Barrera-Mera, 1976) have shown a close dependence between ERG circadian activity and the integrity of neurohemal system-cerebral ganglion sinus gland of the crayfish P. bouvieri. It was proposed that the lack of humoral neurosecretions, is the mechanism through which changes in ERG circadian oscillations in brainless crayfish are brought about. In this paper we report the effects of surgical removal of the cerebral ganglion on short and long-term ERG activity. Light-dark (L-D) adaptation reactions (60 min each) as well as the retinal shielding pigments (RSP) position were determined in intact and in brainless animals, during both rest (ρ) and activity (α) phases of continuous ERG recordings. In contrast with intact animals the time course of ERG voltage during L-D adaptation consists of one component only, probably due to the lack of the movilization of distal RSP. That is, also observed in these animals. These findings an increase in frequency of ERG oscillations and the change in α/ρ ratio value seen in these preparations suggest a central modulatory influence on short and long-term ERG activity in these animals.

526 LACK OF CENTRIFUGAL EFFECTS ON SPEED OF PHASE SHIFTING IN THE APLYSIA EYE. Gene D. Block and Terry L. Page*. Hopkins Marine Station, Stanford University, Pacific Grove, CA 93950.

The eye of Aplysia contains a circadian pacemaker (Jacklet, 1969). This oscillator is entrainable by light both in vitro, when isolated in culture medium (Eskin, 1971), as well as in vivo (Jacklet, 1969). However, there are differences in the speed of entrainment of eyes exposed to light cycles in vivo when compared to exposure in vitro (Eskin, 1971). Enhanced rates of entrainment in vivo might be due to neural or hormonal factors absent in vitro or might reflect deficiencies in the culture medium. The assistance of extraocular neural inputs in phase shifting the ocular rhythm is made plausible by the presence of efferent fibers in the optic nerve (Eskin, 1971). In order to evaluate whether efferent fibers are responsible for enhanced entrainment rates 16 Aplysia were placed on white light cycles (Light:Dark, 12hr:12hr) for 5-7 days. One optic nerve was then surgically cut and the Aplysia placed back on the same light schedule. After 2 additional days the intact and denervated eyes of 4 Aplysia were removed and recorded to ensure that both eyes of the operated animals were in steady-state entrainment. At this time the light cycles were phase advanced by 10 hr. Aplysia were sacrificed at the end of each successive photoperiod and recordings of optic nerve activity made by means of suction electrodes attached to the optic nerves of eyes maintained in filtered seawater at 15.0 + .5°C. in constant darkness.

The results indicate that efferent information does not influence entrainment speed. At the end of the first photoperiod of the advanced light cycles the mean shift for intact eyes was 3.5 hr and 3.6 for denervated eyes when compared to the unshifted controls. After the second photoperiod the mean shift was 9.0 hr for intact and 9.0 hr for denervated while after the third photoperiod, 10.3 hr for intact and 11.5 hr for denervated eyes. There was no additional advance after the 4th photoperiod indicating the pacemakers were in steady state. Additional Aplysia with both optic nerves intact showed similar entrainment rates. Taken together, these results demonstrate that differences in entrainment rates in vivo and in vitro are not due to efferent neural inputs via the optic nerve. Supported by N.I.A. AG00490-02 and N.I.H. IF32NS5035-02.

527 HOURS-LONG INHIBITION PRODUCED BY PEPTIDE-SECRETING BAG CELLS OF APLYSIA, INCLUDING INHIBITION OF CELLS CONTROLLING INKING BEHAVIOR. Philip Brownell* and Earl Mayeri. Dept. Physiol., Sch. Med., UCSF, San Francisco, CA 94143

Previous work indicates that a peptide released from the neuroendocrine bag cells induces egg laying in intact animals. In the isolated abdominal ganglion, bursts of bag cell spike activity lasting 5-40 min can be triggered by local electrical stimulation. We have found that bag cell activity triggered in this manner produced several types of neural changes, both excitatory and inhibitory, that lasted several minutes or hours. Slow inhibition, the most widespread type of change, occurred in 21 of the identified cells we surveyed, including the giant cell R2, white cells R3-R14, the left upper quadrant cells L2-L6, and ink-gland motorneurons L14 A,B & C. It also occurred in several unidentified cells on the ventral surface of the ganglion.

We have begun a detailed study of bag cell-induced slow inhibition in two of these cells, L3 and L6. Hyperpolarization of L3 and L6 typically began 2-8 sec after onset of bag cell activity, and reached a peak amplitude of 10 to 20 mV within 20 sec. For 30 min to 3 hr or more thereafter,both the burst rate and average firing rate of these burster neurons were decreased. The inhibition was (1) contingent on bag cell activity and not on the electrical stimulus that triggered bag cell activity; (2) the response was inverted when the cells were hyperpolarized by direct current injection; (3) the reversal potential was near -80 mV, the potassium equilibrium potential; and (4) the response was not attenuated by tetraethylammonium and curare at concentrations that blocked cholinergic inhibition of these cells. These results strongly indicate that the bag cells produce slow inhibition that involves an increase in membrane conductance to potassium ions. Since bag cells release peptides when they are activated, it is possible that the slow inhibition is mediated by a peptide.

Slow inhibition that occurs in the ink-gland motorneurons L14 A, B&C is similar to that described above. These cells are part of a well-defined neuronal circuit controlling a fixed act, the all-or-none release of ink from the purple gland (Carew and Kandel, JNP, May 1977). Our results predict that in intact animals the threshold for inking is elevated during egg-laying. Thus bag cell activity that produces inhibition of the ink-gland motorneurons might serve to suppress one stereotyped behavior, inking, at the same time bag cell activity induces another behavior, egg laying.

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528 DIFFERENTIAL SENSITIVITY OF APLYSIA AXONS TO PHARMACOLOGIC AND IONIC MANIPULATIONS. Hugh Bryant, Anatomy Department, School of Medicine, UCLA, Los Angeles, California 90024.

The axons of cells R1 and R2 are the largest in the Aplysia right pleurovisceral connective (PVC), and their extracellular field potentials can be readily recorded and distinguished one from the other. This preparation, therefore, offers the possibility of studying the differential responsiveness of these axons to various experimental manipulations. The pleural-pedal ganglion, right PVC and visceral ganglion were mounted in a multi-compartment chamber. Stimulation and recording in oil were accomplished on either side of a 2 cm compartment which contained the experimental medium. For example, in a Na^+-deprived medium (40-50%) the axon of R2 does not conduct in an all-or-nothing fashion but conducts with decrement (Bryant and Decima, 1976, Soc. for Neurosci. Abst.). R1, on the other hand, conducts uniformly until the Na^+ is reduced to approximately 20-30% of its normal value, at which point it also begins to conduct with decrement. The above conclusions are true only for stimulus frequencies below 2-4/sec. At higher frequencies (5-10/sec), R2 paradoxically resumes uniform conduction, and R1 begins to conduct with decrement (even with 40-50% normal Na^+). Following a tetanus in a 40-50% Na^+ medium, the original decrement in R2 is augmented for several minutes, whereas R1 quickly recovers and conducts uniformly. In a "low Ca^{++}, high Mg^{++}" solution whichpurportedly blocks chemical synaptic transmission (Byrne et al., 1974, J. Neurophysiol. 37, p. 1041), conduction in R2 and several smaller axons occurs with decrement. Providing stimulus frequencies are kept low, R1 and still other small, unidentified axons will continue to conduct uniformly. This medium is, in fact, also a Na^+-deficient medium (~46%) and would be predicted to cause conduction with decrement in some, but not all, axons (see above). Its use in Aplysia to separate synaptic from non-synaptic neuronal activation is, therefore, seriously compromised. Finally, the R1 and R2 axons also have different sensitivites to xylocaine. Although the precise concentrations which cause R1 and R2 to conduct with decrement vary slightly from one preparation to another (10-30mM), R2 is always depressed at lower concentrations than R1. Whether or not R1 or R2 conduct uniformly or with decrement during local anesthesia depends on several factors in addition to anesthetic concentration, most notably time of exposure and stimulus frequency. These results: 1) add further support to the classical concept of decremental conduction as the proper description of conduction in narcotized peripheral nerve; 2) demonstrate similarities between conduction in narcotized nerve and certain synaptic phenomena; 3) suggest caution in the generalization of results from one preparation (i.e., cell) to another. Supported by NIH grant MH 10083.

529 HABITUATION OF CEREBRAL NEURONS MEDIATING TENTACLE RETRACTION IN THE LAND SLUG ARIOLIMAX. Christopher Y. Chan* and Stacia Moffett (SPON: F. A. Young). Dept. Zool., W.S.U., Pullman, WA 99164.

The tentacles of Ariolimax columbianus are inverted by tentacle retractor muscles when the slug experiences a novel or aversive stimulus. We have studied cerebral neurons that mediate retraction of the major tentacles in semi-intact preparations and in the isolated central nervous system. The efferent units that mediate the retraction response are found in the cerebral retractor nerve (CRN). Because the large efferent units are few in number (1-3 on each side) and distinguishable by characteristic spike heights, the response of each cell can be quantified in extracellular recordings.

Repetitive electrical shocks delivered to the minor tentacle nerve or other cerebral nerves cause an exponential decline in the number of action potentials reflexly activated in the efferent units. The reflex response recovers spontaneously with rest and shows dishabituation and habituation of the dishabituating stimulus pathway. No generalization of the habituation from one pathway to another has been found. The interval that produces the most rapid habituation is around 45 to 60 sec., which is approximately the time required for the reflex to be completed and the retractor muscle to relax. Short stimulus intervals, as well as high stimulus strengths tend to produce sensitization of the response.

We have identified two symmetrically positioned cerebral neurons as retractor efferent units on the basis of one-to-one correspondence of action potentials in the soma and CRN, and antidromic action potentials following stimulation of CRN. Intracellular recordings from these neurons show that they are reflexly activated by inputs from all cerebral nerves. The response habituates and can be dishabituated in a manner similar to that found in extracellular recordings. Sensitization is also evident as an increase in the general level of activity of these neurons. Each neuron is the dominant unit in the ipsilateral tentacle retraction reflex. When the neuron is depolarized to fire action potentials, no effect of its activity is recorded on the contralateral CRN. We are currently studying the mechanism of habituation in these neurons and attempting to identify other elements in their reflex pathways.

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530 MUTATIONS AFFECTING THE FLIGHT NEUROMOTOR SYSTEM IN DROSOPHILA John C. Coggshall*, (SPON: William Pak). Dept. Biol., Yale Univ., New Haven, CT 06520

An extensive, ongoing, mutagenesis has been conducted yielding a large number of flightless mutants, primarily on the X-chromosome.

The technique of fate-mapping using mosaic flies has been used to distinguish between mutants with primary defects in the nervous system as opposed to primary defects in the muscles. The incorporation of an autonomous, external, marker for the nervous system, namely a temperature-sensitive paralytic, has been incorporated into the mosaic generation system, thus allowing the detection of internal mosiacism within the nervous system without resorting to the use of internal enzyme markers requiring tedious, time consuming, histochemistry.

Results suggest that two general classes of neural mutants can be distinguished; functional mutants and neurotrophic mutants.

Functional mutants appear to have a defect in the nervous system that disrupts the functioning of the nervous system itself. Of five X-chromosome flightless mutants which have been fate-mapped, three have been shown to have defects in the nervous system. Electrophysiological, anatomical, and behavioral data suggest that these are indeed functional mutants. The other two mutants have wing and muscle defects.

A neurotrophic mutant is defective in a function that is necessary for the development and/or maintenance of a particular set of muscles. A potential neurotrophic mutant on the third chromosome has been studied. The phenotype consists of the elimination of one specific set of flight muscles, the dorsal longitudinals, in the adult. Mosaic data indicate that the primary defect is in the nervous system.

531 IN VIVO RECORDING OF REFLEX ACTIVITY FROM LOBSTER CLAW MOTOR NEURONS. Walter J. Costello and Fred Lang, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543.

Crustacean neuromuscular systems possess a large repertoire of synaptic activity. Depending on the neuromuscular properties there can be summation, facilitation, or anti-facilitation. A salient feature of these responses is the fact that patterned pre-synaptic stimuli can be more efficient in eliciting motor responses than unpatterned (equally spaced, constant frequency) stimuli. We have investigated the normal motor pattern of the fast and slow axons to the claw closer muscles by recording intracellularly from muscles of restrained animals.

First, using isolated claws we determined the distribution of the fast and the slow excitor axons to the closer muscle of the cutter and the crusher claws in the lobster. Preliminary results reveal that specific areas of the muscle are innervated by only one of the two single motor axons. Thus it is possible to observe the activity of each axon by monitoring a known area.

These results were utilized to ascertain in vivo reflex activity of the neuromuscular system in the lobster claw closer muscle. Small holes were drilled in the claws of restrained animals; microelectrodes were inserted into the holes to record from individual muscle fibers. Reflex activity was evoked by stimulation of the sensory hairs on the dactyl and propus. Claw closure was accompanied by one or a few excitatory postsynaptic potentials (EPSP's) in the fast motor axon. A fast twitch was accompanied by a pair of EPSP's with an interval of less than 9 msec. The second EPSP was greatly facilitated and usually evoked a graded membrane response.

Slow claw closure was accompanied by a burst of EPSP's from the slow motor axon. These occasionally contained paired EPSP's with interpulse intervals of 9-15 msec but no active membrane responses were observed.

Therefore it appears that claw closure can be achieved by only a few impulses from the fast motor axon. When these are patterned into paired pulses, two closely spaced stimuli give sufficient synaptic facilitation to evoke an active membrane response which will, in turn, result in a rapid twitch.

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532 MEASUREMENTS OF OXYGEN PARTIAL PRESSURE AND SINGLE-UNIT ACTION POTENTIALS IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. P.E. COYER *(SPON: R.J. Bradley). Department of Neurology, University of Alabama Medical Center, Birmingham, Al 35294.

Intracellular determinations of oxygen partial pressure and simultaneous recordings of single-unit action potentials have been obtained from identifiable neurons in the isolated ganglion of Aplysia californica with polarographic current measurements using gold microelectrodes. Previous combined oxygen level and action potential recording techniques relied on inferential spectrophotometric measurements from heme-protein-containing cells during penetration with intracellular microelectrodes (Chalazonitis and Arvanitaki, 1970). In those experiments many neurons were reported to respond to hypoxia by a decrease in pigment oxygen saturation usually followed by an increase in spike frequency and sometimes by membrane depolarization. Both membrane potential monitoring and single-unit action potential recording are being undertaken with 3M KCl -filled pipettes and gold microelectrodes from the same identifiable neuron to ascertain the effects of hypoxia on membrane potential and spiking patterns.

This report concentrates on intracellular oxygen level determination from unpigmented neurons (R_{15}, R_8 and L_7) and that of supporting tissue surrounding them. Beating and bursting pacemaker activities have been attributed to single neurons in isolation from the ganglion by several other workers. I report intracellular oxygen levels which have been obtained from ganglionic neurons in conjunction with their characteristic patterns of spiking activity. Attempts are being made to experimentally manipulate the ganglion tissue level in vitro to those reported in vivo. It is hoped that once the link between the level of intracellular oxygen supply and single-unit activity is known that further parameters underlying oxygen diffusion into the interior regions where these identifiable neurons are found can be established.

533 ACQUISITION AND RETENTION OF A LONG-TERM BEHAVIORAL CHANGE IN *HERMISSENDA CRASSICORNIS*. T. Crow and D. Alkon*. Sect. on Neur. Systems, Lab. of Biophys., NINCDS, MBL, Woods Hole, MA 02543.

The nudibranch mollusk *Hermissenda crassicornis* is attracted to a light source. It has been shown previously for groups of animals that this attraction to light can be modified by the paired presentation of a light stimulus and a rotational stimulus (Alkon, 1974). The number of animals reaching and entering a spot of light is significantly smaller for groups that received paired stimulation immediately before testing as compared with groups receiving light or rotation (Alkon, 1974). We now have shown that this behavioral change to a light stimulus is long-term, lasting several days. The effects of paired and single stimulus presentations were assessed at the end of each training session by examining changes in the animals response latencies to enter a light spot. Four groups were trained and tested for seven consecutive days. One group received 40 trials each day of light (30 seconds) paired with rotation (30 seconds). An additional group received 15 trials each day of the same light and rotational stimuli. Control groups received 40 or 15 trials of light and 40 or 15 trials of rotation. The groups that received paired light and rotation took significantly longer to reach the light spot as compared with the respective control groups ($p<.001$). In addition, there was a significant increase in response latencies over the seven training days for the groups that received paired stimulation ($p<.01$) while this was not observed for the respective control groups. We next investigated the retention of this behavioral change. Subjects received a pre-test to light to establish baseline response latencies. One group received 50 trials of light paired with rotation for three consecutive days. The response latencies to enter the light were measured at the end of training day three. These subjects exhibited significantly longer latencies as compared with their own pre-training baseline ($p<.005$) and the respective control groups that received 50 trials of light or 50 trials or rotation ($p<.005$). The subjects were then divided into two groups, one received a retention test two days following the last training session and the other received a retention test four days after the last training session. The experimental groups showed significant retention at two days and four days ($p<.005$) as compared with the control groups and baseline response levels. Two additional retention tests examined changes in light responsiveness on two subsequent days following retention tests 2 and 4. These results revealed that response latencies gradually declined such that the latencies were not significantly different from controls or baseline levels on the last retention day. These results suggest that the changes in response latencies following paired stimulation are long-term and resist repeated testing.

534 DESCENDING CONTROL OF COCKROACH GIANT FIBERS DURING WALKING Darryl L. Daley* (SPON: F. Delcomyn). Program in Neural and Behavioral Biology, Univ. of Illinois, Urbana, IL 61801.

The ventral nerve cord (VNC) of the American cockroach contains about 16 giant fibers (GF's), commonly associated with some aspect of escape behavior. Delcomyn (J. Insect Physiol. 22:1223, 1976), monitoring activity from the VNC of a tethered cockroach capable of normal walking, has found certain giant fibers are activated during walking. This finding was unexpected because activity in GF's had been thought to occur mainly before and possibly during the escape run, and not during slow walking.

To further investigate GF activity during walking in the cockroach, experiments have been performed using tethered walking preparations. Giant fiber activity was monitored en-passant with suction electrodes from the connectives of the VNC. The results demonstrate the existence of at least two distinct populations of GF's under descending control during walking. The first population consist of those GF's shown by Delcomyn to be active during walking but near silent during rest. Activity of a second population of giants can be evoked by presenting the animal with 10-12 Hz auditory clicks. The activity of these GF's is inhibited during walking, coincident with the activation of the first population of GF's. Murphey and Palka (Nature 248:249, 1974) have described a similar inhibitory influence on cricket giant fibers. Both excitatory and inhibitory influences on giant fiber activity are unilateral and appear to originate in part from thoracic or higher central nervous centers. After establishing the two modes of GF control active during walking, experiments were done to examine these influences upon single GF's. The results of intracellular recordings from identifiable GF's during walking in tethered animals allowed specific inhibitory or excitatory influences to be uniquely associated with individual giant fibers.

The findings reported here support the ideas that giant fibers are under two separate modes of descending control during walking and that they do not all function exclusively in escape. This research was supported in part by RIAS NSF grant SER 76-18255, NIH grant NS12142 to F. Delcomyn, and HEW PHS grant GM-1076 to the author.

535 SYNAPTIC GROWTH IN A CRUSTACEAN MUSCLE. Richard A. DeRosa and C. K. Govind. Scarborough College, University of Toronto, West Hill, Ontario, Canada M1C 1A4.

The absence of a terminal molt in a lobster allows its muscle fibers to continue growing in length and diameter throughout the animal's life span. If it is desirable to have uniform depolarization of the muscle fiber at all stages of growth, then changes must occur in their synaptic properties, electrical properties, or in both. These changes have been examined in the proximal head of the limb accessory flexor muscle of the lobster by comparing two distinct age groups, viz., adult (1 lb) and old (6 lbs) lobsters.

During growth of the muscle fiber from adult to old lobsters the input resistance (R_{in}) decreased according to the fiber diameter$^{3/2}$. However, postsynaptic potentials and facilitation ratios were similar for comparable muscle fibers in both age groups and were independent of R_{in}. Furthermore, muscle fibers in both age groups have similar values for membrane time constant, specific membrane resistance, membrane capacitance and sarcoplasmic resitivity. Consequently, the equivalent levels of depolarization seen in adult and old lobsters must be due to increased quantal output (m) in older lobsters. The quantal output increased to 3.4 in old lobsters compared to values of 2.2 in adult lobsters.

Ultrastructural analysis suggests that the increases in m values from adult to older synapses are due to increases in total synaptic area and dense body accumulations. Also it was observed that the structural properties of sarcomere length, A-band length and total sarcomere number were still growing in the oldest of lobsters examined (12 lbs).

It is concluded that the accessory flexor muscle maintains similar levels of depolarization throughout its life span. This is accomplished by increasing the quantal output rather than changing the electrical properties of the muscle fiber.

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536 IDENTIFICATION OF HORIZONTAL AND VERTICAL MOVEMENT DETECTION SYSTEMS IN INSECTS. Hendrik E.A.Eckert.USC, Dept.Biol.Sci.,Los Angeles. CA 90007.

The iontophoretic injection of the fluorescent dye Procion Yellow has yielded the anatomical identification of two groups of neurones in the flies Phaenicia Sarcophaga,which respond selectively to either horizontally or vertically moving patterns. These cells are located in the third optic ganglion (lobula plate) with their axons extending into the protocerebrum (homolateral elements)or crossing the midline and extending to the contralateral side of the brain and contralateral lobula plate (heterolateral element)

Vertically sensitive fibers were split into two groups:(1) 9 giant homolateral elements (V-cells) respond with a depolarizing DC-membrane potential shift (mps) to downward, and with a hyperpolarizing mps to upward movement of a pattern. Elements with anterior receptive fields in the eye exhibit only this response behaviour; cells with lateral and posterior receptive fields possess an additional sensitivity to progressive pattern motion (front to back) by responding with a depolarizing mps; regressive motion elicits a hyperpolarizing mps. (2) Another element VS1 which crosses the midline of the brain (hetrolateral element) generates spikes and exhibits a preference sensitivity to downward movement and a slightly weaker response to progressive motion. It is believed to be postsynaptic to the 9 V-cells on anatomical and physiological grounds.

Horizontally sensitive cells are also split into two groups:(1) 5 giant cells of two different types, one type (2 centrifugal horizontal cells) only conducting discrete excitatory and inhibitory graded potentials, the other (3 H-cells) conducting graded potentials and action potentials, the latter potentials believed to be the output of these cells controlling the yaw torque response via descending neurones.Both types are homolateral elements with binocular receptive fields.(2) One heterolateral spike conducting element was identified responding to ipsilateral regressive pattern motion with strong excitation and to progressive pattern motion with inhibition over the background spike frequency. Response properties of this cell resemble those of the class IIa1 motion fibers studied by extracellular recording techniques.

537 PHOTOSENSITIVITY OF TONIC FLEXOR MOTONEURONS OF THE CRAYFISH ABDOMEN. Donald H. Edwards, Jr.* (SPON: N. A. Newby). Dept. of Biol. Sci., Stanford Univ., Stanford, CA 94305

Light incident on any one of the five rostral ganglia of the crayfish (Procambarus clarkii) evokes an increase in the spontaneous discharge frequency of the tonic flexor motoneurons which have cell bodies in the illuminated ganglion. The spike frequency of the tonic extensor inhibitor of the same segment also increases in response to the light. The tonic extensor motoneurons are unaffected by light stimuli. Stimulation of the caudal photoreceptor neuron with light directed at the sixth abdominal ganglion evokes no change in the discharge of rostral tonic flexor motoneurons. The tonic flexor motoneurons are most sensitive to blue (436 nm.) and green (546 nm.) monochromatic light and less sensitive to longer wavelengths. Dark adaptation increases the sensitivity of the light response, while light adaptation, or repeated brief exposures to light stimuli, decreases the sensitivity. When thoroughly dark adapted, blinded intact animals are consistently observed to flex their abdomens in response to light directed at the ventral side of their extended abdomens.

538 CHEMOSENSORY NEURONS IN APLYSIA. Dennis G. Emery and Teresa E. Audesirk*. Dept. Zoology, Iowa State University, Ames, IA 50011 and U. of Washington, Friday Harbor Labs, Friday Harbor, WA 98250.

Behavioral and physiological studies have shown that Aplysia makes use of chemical cues in several activities, including food finding, feeding, and possibly mating. Behavioral observations and ablation studies indicate that the chemoreceptors involved are located in the anterior tentacles, rhinophores, and the circumoral area (lip). These organs were examined with light and electron microscopy to identify and characterize the sensory neurons. The rhinophores and tentacles have dark-pigmented grooves that are sensitive to chemicals. The epithelium of these grooves and of the lip is made up of ciliated columnar cells. Below the epithelium are ganglia containing a few to several dozen neurons each. A slender dendrite arises from the distal pole of each neuron, extends through the epithelium and terminates in a tuft of cilia at the surface. The dendrites have pale cytoplasm containing many microtubules and are thus easily identified among the epithelial cells. Each sensory ending bears up to thirty cilia which may arise from a shallow, subterminal invagination. Axons arise from the proximal end of the ganglion and merge to form major nerve trunks. On the basis of light microscopy there appear to be two morphological types of receptors. One type has only one or two cilia while the other has many. This observation has not been confirmed with electron microscopy, but physiological studies indicate the presence of both mechanoreceptors and chemoreceptors in these organs. This might account for the two morphological types among the sensory cells.

The arrangement of the sense cells in discrete subepithelial ganglia might make it possible to record directly from the sensory ganglia with microelectrodes. Such recordings have been made from the nerves, and from identified central neurons to which they project. It may be possible to study electrical activity in this system at the receptor, nerve, and central neuron levels simultaneously. This sensory system might thus be useful for studying both sensory processes and the role of sensory neurons in behavior.

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539 ENTRAINING A CIRCADIAN RHYTHM FROM THE ISOLATED EYE OF APLYSIA: THE INVOLVEMENT OF CHANGES IN MEMBRANE POTENTIAL. Arnold Eskin, Dept. Biol., Rice Univ., Houston, TX 77001.

The circadian rhythm of nerve activity from the Aplysia eye can be photoentrained in vitro. For entrainment, the environmental information must be absorbed by a photopigment, coded into a form which can be propagated, and then decoded by the circadian oscillator (CO) resulting in a phase shift of the rhythm. The information may be translated into a transmembrane potential change as it flows from the environment to the CO, or, the CO may be associated with photoreceptors and receive information directly from the photochemical reaction. Entrainment information seems to be translated into changes in membrane potential since there is a correlation between those treatments which blodk phase shifting by light and the effects of these treatments on the ERG (Eskin, J. Neurobiol., in press). Also, a depolarizing stimulus, increased K_o^+ (HiK), produces a response curve with both advance and delay phase shifts (Eskin, J. Comp. Physiol. 80:353, 1972).

To further explore the involvement of changes in membrane potential and the CO, we investigated whether other types of depolarizing stimuli could phase shift the rhythm. Strophanthidin (ST), a Na-K pump inhibitor, and Li^+, which is transported poorly by the Na-K pump, were used. Four hr. treatments of either 100% LiCl:0 NaCl or 50% LiCl:50% NaCl caused phase shifts in the rhythm, whereas treatments of 10% LiCl:90% NaCl did not. A phase response curve which contained only delay phase shifts was obtained using 100% LiCl treatments. The response curve obtained using ST (4 hr., $6x10^{-7}$M) contained both advance and delay phase shifts. An inward Na^+ flux seems to be involved in the ST produced phase shift since the effect of ST was abolished by treating the eye with ST ($1.2x10^{-6}$M) in the presence of a low Na^+ (35mM) solution. Release of neurosecretory substances does not seem to be involved since ST phase shifts were not diminished when the eye was exposed to ST in the presence of a high Mg^{++} (125mM) low Ca^{++} (.1mM) solution.

The qualitative similarity in the HiK and ST phase response curves strengthens the case for a coupling between the membrane potential and the CO. Also, ion concentrations may be important. If so, at least Na^+ and K^+ must be involved since the HiK and ST treatments should produce different changes in each ion. The difference between the response curves of LiCl and of HiK and ST point to a different effect of LiCl on the CO. LiCl may change intracellular concentrations of divalent ions. This might be true since the shape of the LiCl response curve is similar to others obtained using agents known to produce such effects. (Eskin and Corrent, J. Comp. Physiol. in press). Supported by NSF Grant BNS75-23452.

540 MODIFICATION OF TACTILE RESPONSES IN APLYSIA FOLLOWING FOOD CHEMICAL PRESENTATION. Steven M. Fredman and Behrus Jahan-Parwar. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Mechanical and chemical stimuli elicit very different behaviors in Aplysia. Tactile stimulation of a tentacle usually causes a withdrawal response. Chemical stimuli such as seaweed extracts (SWE) elicit very different behavior such as head waving and mouth opening which are part of the feeding sequence. Tactile stimuli presented after a chemosensory stimulus produces an altered response. Instead of withdrawal there is an orienting response consisting of bending toward the stimulated side. To determine how the mechano-and chemosensory modalities are distinguished and how the different behaviors they mediate are controlled, the responses of neurons in the identifiable A and B clusters of the cerebral ganglion were examined. The isolated CNS of Aplysia with the anterior tentacles attached was used. Each tentacle was placed in its own sealed compartment. Tactile stimuli were presented by a solenoid. Chemical stimuli consisted of 1 ml of SWE presented 1 cm from the tentacle. An interval of 20-30 min was allowed between tests which were each preceded by a control stimulation with seawater.

Tactile stimuli produced phasic excitatory responses in the B neurons. The A neurons were usually inhibited at a longer latency. Stimulation with SWE produced strong tonic firing in the B neurons. Both excitatory and inhibitory A neuron responses were obtained, all at longer latencies. A neuron responses to tactile stimulation were altered following chemosensory stimuli. After the presentation of SWE a subsequent tactile stimulation caused A neuron firing. It was not necessary to have SWE present. These results could be obtained even after the SWE had been washed out. This suggests that some type of gating or arousal mechanism is involved. The excitatory A neuron responses appear to be the result of a polysynaptic pathway from the B neurons to the A neurons. Strong firing of the B neurons might raise the level of excitation of the interneurons involved sufficiently to produce A neuron firing, or if below threshold, raise the level of excitation enough that a subsequent tactile stimulus triggers the A neurons. Since the A neurons appear to be motor neurons producing body movements, their activity may be a correlate of the orienting response. This possibility and the mechanisms underlying the modification of A neuron responses are being investigated.

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541 ON THE MECHANISMS OF MIGRATION OF PIGMENT GRANULES OF THE RETINULA CELLS OF THE CRAYFISH. Eugenio Frixione*†, Victor Tsutsumi* and Hugo Aréchiga. Depts. Neurosc. and Physiol. CIEA, IPN, and Dept. Electr. Microsc. Reg. Nac. Anat. Patol., SSA, México, D.F.

The retinula cells of the compound eye of the crayfish contain a set of accessory pigment granules, the position of which is a function of the intensity of illumination. In a fully dark-adapted eye, the granules migrate longitudinally away from the cell bodies and accumulate along the axons, whereas the opposite occurs under light-adaptation. The mechanism underlying this photomechanical response is at present poorly understood. In isolated eyestalks or retinas of the crayfish Procambarus bouvieri, the position of pigment granules was determined after fixation at different times of light- or dark-adaptation. The time course of granule migration was quite different under light than in darkness. While the light-induced migration proceeds in a sigmoidal fashion, being complete in about 30 min, depending on light intensity, the migration in darkness shows two distinct phases: an initial stage, during which the granules move from the distal regions of the cell body to the middle zone, with a time constant of 6 min, ends on a short plateau from which a further rapid movement clears up the pigment from the proximal regions of the cell body and takes the granules deep into the axon. The full dark-adaptation is over in about 45 min. The migration in either direction is temperature-dependent with a Q_{10} of approximately 2.0 Hypoxia and metabolism-blocking agents, like cyanide (2 mM), prevent the second stage of dark-adaptation and induce distal migration in the absence of light. The effect of oxygen deficiency has shown to be reversible. Electron microscopy reveals a close correlation between the intracellular arrangement of pigment granules and a central bundle of microtubules present in the middle and proximal regions of the cell body but being most prominent in the axon. Treatment for 2 hs with 10 mM colchicine or 90% D_2O blocks the second phase of migration in the dark but, although by itself does not promote distal migration, it does not interfere either with the movement towards the cell body induced by exposure to light.

These results suggest the existence of two different mechanisms of migration of pigment granules in the photoreceptor cells of the crayfish, dark-adaptation showing greater metabolic requirements than light-adaptation and being dependent for its completion upon a microtubule-involving mechanism of granule translocation.

†CONACyT Fellow, México

542 INTRACELLULAR ANALYSIS OF PRESYNAPTIC INHIBITION IN THE CRAYFISH CLAW OPENER. Paul A. Fuchs, Neuroscience Program, Stanford University, Stanford, California, 94305.

Presynaptic inhibition of excitatory transmission in the claw opener neuromuscular junction proceeds by a reduction in the number of quanta released by an action potential (Dudel, J., and S.W. Kuffler, 1961c, J. Physiol. 155:543.). The mechanism by which this reduction comes about is not entirely clear. In other systems, such as the spinal cord and the tactile afferents of crayfish, presynaptic inhibition is seen as a depolarizing potential in the excitatory terminals, which in some manner reduces the ability of the excitatory spike to release transmitter. Intracellular recording from the excitor axon on the ventral surface of the opener muscle reveals hyperpolarizing IPSPs in response to action potentials in the inhibitor. These IPSPs are 100 - 200 uV in amplitude and approximately 80 msec in duration. They are presumably a summed potential from the many synaptic contacts of the inhibitor onto the excitor. Thus, in contrast to primary afferent depolarization, presynaptic inhibition at this synapse is mediated by a hyperpolarizing potential.

The action potential of the excitor axon is typically 100 uV in amplitude, overshooting the 70 mV rest potential. The action potential is followed by a depolarizing after potential of 5 to 10 mV amplitude and approximately 80 msec duration. When the excitor axon is stimulated at 40 Hz., each spike rides on the afterpotential of the previous spike, producing a depolarizing shift of several millivolts during the train. When excitor action potentials are elicited during stimulation of the inhibitor, the depolarizing after potential is partially shunted by the IPSPs, and trains of excitor spikes do not shift up to the same extent. Finally, during trains of excitor spikes, the duration of the action potential increases by 20% (for 10 spikes at 40 Hz.). Presynaptic inhibition has little effect on this process. Observed postsynaptically, presynaptic inhibition reduces ongoing transmitter release but has little effect on the progress of facilitation. This observation, taken in conjunction with those above, prompts further investigation into the role of use-dependent axonal conduction in the processes of facilitation and presynaptic inhibition at this neuromuscular junction.
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543 LENGTHENING THE PERIOD OF A CIRCADIAN RHYTHM IN PROCAMBARUS BY HEAVY WATER. Beatriz Fuentes-Pardo and F. Félix-Durán*. Depto. de Fisiología, Fac. de Medicina, UNAM. México 20, D.F. MEXICO

Deuterium oxide lengthens the period of several endogenous circadian rhythms. Heavy water also produces a reversible slowing of several biological rhythms with periods in the millisecond range. In order to obtain information about the role of this substance on the clock mechanism underlaying the circadian electroretinographic rhythm, we have obtained long-termed records of the ERG of both, intact crayfish and isolated eyestalk of crayfish which exhibit high frequency cycles (Sánchez and Fuentes-Pardo, 1976). Heavy water lengthens both, the circadian and the ultradian rhythms with a dosage dependent character. Since the high frequency oscillations have been taken as the expression of a splitting of the circadian rhythm (i.e. the loss of sinchrony between at least two oscillators), the experimental results suggest the possibility that the changes observed in the circadian rhythm associated with the presence of deuterium oxide, are a consequence of the changes produced on the ultradian cycles.

544 SENSORY INPUTS TO A MOLLUSCAN FEEDING MOTOR PROGRAM. Alan Gelperin, Joseph Jin Chang and Stephen C. Reingold. Dept. of Biology, Princeton University, Princeton, N.J. 08540

A preparation of the lips, cerebral ganglia and buccal ganglia of the terrestrial slug Limax maximus has been developed which allows us to quantitatively elicit bouts of feeding motor program (FMP) by applying defined chemical stimuli to lip chemoreceptors. Expression of the FMP was monitored by extracellular recording of efferent activity in buccal nerves and intracellular recording of activity in buccal motoneurons and cerebral interneurons. The two autoactive salivary burster neurons provide a particularly clear monitor of the FMP as their bursting is rigidly phase-locked to the protraction-retraction cycle during feeding (Prior and Gelperin, J. Comp. Physiol. 114: 217, 1977).

A variety of pure chemical stimuli and food plant extracts have been tested for the vigor and reliability of the motor response which they elicit. The most effective stimuli found to date are extracts of the most attractive food plants (mushroom, potato, carrot). Application of secondary plant substances such as nicotine sulfate (6% wt/vol), quinine hydrochloride (1% wt/vol) and colchicine (0.01 M) give weak feeding responses when first applied. The feeding response to colchicine decrements rapidly after 2-3 applications. Colchicine can suppress a feeding response previously triggered by an attractive food plant extract. Rebound excitation of the FMP is observed when colchicine is removed.

One minute of chemical stimulation elicits a bout of FMP which can last for 3-4 minutes. Variations in the nature or concentration of the chemical stimulus affect the peak frequency of biting, the mean frequency of biting and the duration of the FMP episode. The basic coordination of activity phases of identified motoneurons is not altered significantly.

In addition to chemosensory stimuli which affect the feeding motor program, mechanical stimuli from the buccal mass may also alter on-going feeding rhythms. Bite frequency is significantly greater when an animal feeds on soft food than on hard food. In a lip-brain preparation in which the buccal musculature is left intact and innervated, we have found sensory units in the buccal mass which are responsive to deformations of buccal muscles. These units also fire rhythmically in phase with contractions of the buccal mass during bouts of feeding.

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545 INTRACELLULAR Ca^{2+} CHANGES IN A MOLLUSCAN PACEMAKER NEURON MEASURED WITH ARSENAZO III. A. L. F. Gorman and M. V. Thomas* Dept. Physiol., Sch. Med., Boston U., Boston, MA 02118

Changes in free intracellular Ca^{2+} were measured in the neuron R-15 in the abdominal ganglion of Aplysia during spontaneous bursting pacemaker activity and during depolarizing voltage clamp steps. The soma was injected with the Ca^{2+} sensitive dye Arsenazo III and changes in dye absorbance were used to detect changes in $[Ca]_i$. We find that $[Ca]_i$, but not $[Mg]_i$ increases during each depolarizing pacemaker cycle. Our results suggest that the increase in $[Ca]_i$ during the pacemaker cycle occurs as a result of Ca^{2+} influx rather than from release from intracellular stores. Part of the influx is associated with each of the action potentials during a burst. The change in $[Ca]_i$ occurs in steps coincident with each action potential. A second part of the influx is associated with the slow depolarizing pacemaker wave. The absorbance changes are greatly reduced, but not abolished, when action potentials are blocked by the adding of TTX to the external media and reducing $[Ca]_o$ from 10mM to 1mM. In voltage clamped cells, held at -50mV, step depolarization as small as +5 to +10mV are sufficient to cause a Ca^{2+} influx and thereby increase $[Ca]_i$. This influx is reduced by the addition of 10mM Co^{2+} or Mn^{2+} and abolished by the addition of 1-3mM La^{3+} to the external media. We calculate that the total increase in $[Ca]_i$ during the burst is approximately 5 x 10^{-8}m. (supported by NIH Grant NS11429)

546 NON-SPIKING SYNAPTIC TRANSMISSION BETWEEN SPIKING NEURONS. Katherine Graubard, Jonathan A. Raper* and Daniel K. Hartline. Dept. of Zoology, Univ. of Washington, Seattle, 98195 and Depts. Neuroscience and Biology, UCSD, La Jolla, Calif. 92093

Graded synaptic transmission exists between spiking neurons of the lobster stomatogastric ganglion. These neurons have action potentials which cause IPSPs in their postsynaptic cells; however, it is often possible to demonstrate inhibition in the postsynaptic cells caused by subthreshold depolarization of the presynaptic neurons (Fig. 1). The synaptic connections studied were PD to LP, PD to PE (early PYs), PD to PL (late PYs), and LP to PD. Reversal potentials were similar for graded release and for spike-evoked IPSPs.

When $2X10^{-7}$M TTX was used to eliminate action potentials, the response to a suprathreshold presynaptic voltage step was a two-component (peak-plateau) hyperpolarizing waveform in the postsynaptic neuron (Fig. 2 inset). Both peak and plateau increased in amplitude with increasing presynaptic depolarization (Fig. 2 graph). All the studied cell pairs with strong synaptic connections in normal Ringer displayed graded release in TTX, suggesting involvement of the same synaptic terminals in both types of interaction. PD neurons often had a release threshold more negative than the resting potential (in TTX), so that presynaptic hyperpolarization caused a postsynaptic depolarization by disinhibition.

Preliminary work indicates that hyperpolarizing PL electrotonically hyperpolarizes LP, reducing graded release from LP onto PD. Depolarizing LP causes graded release which hyperpolarizes PD, thereby reducing graded release of PD onto PE.

In addition to spiking, these cells are characterized by large amplitude oscillations in membrane potential (15-20 mV) during normal cyclic activity. The resulting modulation of graded release may contribute to the phasing of units within the cycle.

(Support: USPHS fellowship NS05060; NIH grant NS13138).

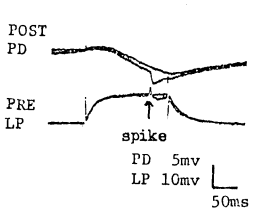

FIG. 1

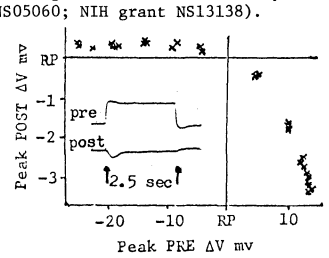

FIG. 2

547 OPTICAL MONITORING OF ACTIVITY IN BARNACLE NEURONS IN RESPONSE TO LIGHT STIMULATION OF THE MEDIAN PHOTORECEPTORS. A. Grinvald* and L. B. Cohen. Dept. of Physiology, Yale University School of Medicine, New Haven, CT 06510.

The changes in light absorption that occur in stained neurons in response to changes in membrane potential have been used to monitor spontaneous activity in cell bodies of the supraesophageal ganglion of Balanus nubilus (Salzberg et al, J. Neurophysiology, in press). We have now used this technique in a preliminary effort to localize ganglion cells which respond to light stimulation of the photoreceptors of the median ocellus. The ocellus and ganglion were in separate sections of a black lucite chamber; the light stimulus (510 nm) and the optical recording of membrane potential (720 nm using the merocyanine-oxazolone dye, NK 2367) did not interfere with each other. Two suction electrodes were used for electrical recording of activity in a root and a connective.

We were concerned about the possibility of pharmacological effects of the dye. (The dye concentration in the incubation solution was 2×10^{-3} molar.) However, in most stained preparations, the electrically recorded off-response to a light stimulus was similar to the response observed in unstained preparations. While such comparisons are difficult because the preparations tended to deteriorate with time, it seemed that pharmacological effects of the dye were not large even when the responding neurons were second or higher order.

In experiments where the ocellus was stimulated and fifteen neurons were monitored simultaneously, most neurons remained silent. Using the simultaneous optical and electrical measurements it was possible to specify the location and axon pathway for those neurons that did participate in the off-response. It would be easier to locate responding neurons if all the neurons were monitored simultaneously instead of just fifteen. We are presently trying to construct such an apparatus.

Supported by grant number NS 08437 from the Public Health Service.

548 HAIR MOVEMENTS, VOLTAGE NOISE AND GENERATOR POTENTIALS IN STATOCYST HAIR CELLS. Yoram Grossman*, Eliahu Heldman and Daniel L. Alkon* (SPON: A. Fein). Section on Neural Systems, Laboratory of Biophysics, NINCDS, NIH, MBL, Woods Hole, MA 02543.

To elucidate mechanisms of mechanotransduction, hair movements, membrane potential fluctuations (voltage noise) and generator potentials were analyzed for hair cells in the statocyst of the nudibranch mollusk *Hermissenda crassicornis*. Scanning electronmicrographs and visualization with Nomarski optics revealed that the statocyst is a sphere consisting of 12-13 cells whose luminal surfaces are covered by motile hairs (12-15 hairs/100 μ^2). The mean movement frequency of free hairs as determined by a stroboscope was 10 Hz (at 20°C) and was reduced to 7 Hz when the hairs experienced the weight of the crystals (statoconia) within the statocyst lumen. Intracellular recordings from hair cells revealed that cells in front of a centrifugal force (produced by a rotating table) depolarized up to 25 mV (with a maximum stimulus of 1.7 g) and showed up to 400% increase of resting voltage noise amplitude. Cells behind the force vector hyperpolarized (max. 12 mV) and the voltage noise was almost eliminated. Both the increase in voltage noise amplitude and depolarizing generator potential were associated with an increase of membrane conductance within a limited range of membrane potential. Increases in voltage noise amplitude and hair cell generator responses elicited by rotation were similarly dependent on the level of membrane potential. Perfusion of the statocyst with zero external sodium eliminated the generator potential and the voltage noise without noticeable effect on the hairs' movements. Voltage noise, generator potentials, and the movement of the hairs and the statoconia were reversibly eliminated by perfusion with 5-10 mg/ml chloral hydrate, a known anesthetic. Lowering the bath temperature from 20° to 10°C and perfusion with 2600 mOsmoles hypertonic solutions reduced the hairs' frequency of movement (25% and 50% respectively) and similarly reduced the voltage noise amplitude. The results of this study demonstrate that the movements of the hairs themselves, although necessary, are not sufficient to produce substantial voltage noise. It is thus the collision of statoconia with the motile hairs which produces most of this voltage noise. The results also indicate that the voltage noise and hair cell generator potential have a common origin: exertion of force on statocyst hairs by the weight of the statoconia. Thus it is suggested that hair cell depolarizing generator potentials arise from summation of depolarizing waves within the voltage noise, in a manner that may resemble the production of photoreceptor generator potentials from summation of voltage noise events associated with quantal photon capture.

549 THE EXTENSION PHASE OF THE CRAYFISH ESCAPE RESPONSE. Grace Hagiwara* and Jeffrey J. Wine. (SPON: Angela C. DiBerardino) Dept. of Psych., Stanford U., Stanford, CA 94305.

Perhaps the most basic patterned activity generated by nervous systems is the sequential activation of antagonistic muscles. We investigated neural mechanisms underlying such coordination in the crayfish 'tailflip' response.

It has long been known that the giant axons of crayfish are command neurons that trigger tail flexion and reextension (Wiersma, J. Neurophysiol. 1947). Flexion is produced directly by monosynaptic activation of flexor motoneurons, but the neural basis of reextension is unknown. We recorded intracellularly in extensor motoneurons and inhibitors to establish the following points: (1) In an isolated abdomen, the only effect of the command neurons on the extensor system is inhibition. (2) Command-derived inhibition operates at three levels: (i) Extensor motoneurons are inhibited at short latency via an unknown pathway; (ii) Extensor muscles are inhibited by the peripheral inhibitor; and (iii) The muscle receptor organs (MROs), which are stretch sensitive proprioceptors in parallel with the extensor muscles, are inhibited via their accessory innervation.

Since no command excitation of extensor motoneurons could be demonstrated, we looked for sensory pathways to the extensor motoneurons. Two were found. (1) The tonic and phasic MROs excite the extensor motoneurons. The connections are chemical and meet most criteria considered to be indicative of monosynaptic transmission. The connections are extensive; at least three of the five extensor motoneurons in each hemiganglion are contacted by the MRO axons, which then bifurcate and make weaker connections with extensor motoneurons in the anterior and posterior ganglia. (2) A polysynaptic pathway to the extensor motoneurons was found that can be activated by any sensory root in the abdominal nervous system. The pathway causes excitation of extensor motoneurons that peaks 25-35 msec after the stimulus and is associated with the discharge of sustained sensory interneurons.

In summary, the results show that although a command neuron impulse can trigger a flexion-extension sequence in an intact animal, only inhibition of extensor motoneurons is seen in an isolated abdominal cord. Excitation occurs as a result of sensory feedback. The source of the polysynaptic sensory input and the contribution of the rostral nervous system to the organization of extension are now being investigated.

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550 EQUILIBRIUM RECEPTORS IN THE COCKROACH ARENIVAGA. H. B. Hartman, W. W. Walthall*, L. P. Bennett*, R. R. Stewart*. Dept. Biol. Sci., Texas Tech Univ., Lubbock, TX 79409.

Pendulous sensilla located on the ventral surface of the cerci of the burrowing desert cockroach Arenivaga are adapted specifically for the detection of changes in the insect's equilibrium. Each sensillum (tricholith) consists of a dense sphere positioned at the distal end of a slender shaft, the latter inserting into an innervated socket. The tricholiths generally occur in pairs, the number varying with the developmental stage of the animal. Adults have as many as eight pairs per cercus.

The information from the tricholiths is integrated by two pairs of giant interneurons in the ventral nerve cord. On roll left, the two interneurons in the right connective are active. The two interneurons in the left connective respond to roll right. Pitch forward evokes activity from the two smaller interneurons of both connectives, whereas pitch backward prompts activity from the two larger units. Rotations between roll and pitch are signalled by variations in the ratio of activity of the two pairs of interneurons. In each instance, the frequency of firing is proportional to the angular displacement. The animal's orientation as related to gravity is therefore determined by which interneurons are active, the ratio of their activity, and the frequency of firing.

Immobilization of the tricholiths on one cercus inactivates the large interneuron on the ipsilateral side and the small interneuron on the contralateral side. If the tricholiths of both cerci are immobilized, the interneurons no longer respond to positional changes.

This is the first report of specific receptors and interneurons responsive to gravity orientation in insects.

551 ELECTRICAL COUPLING AND BILATERAL INTERACTIONS OF BAG CELLS IN APLYSIA. J.T. Haskins and J.E. Blankenship. Marine Biomedical Inst. and Dept. Physiol. and Biophysics, Univ. Tex. Med. Br., Galveston, TX 77550.

Two bilateral clusters of bag cells in the abdominal ganglion of Aplysia release an egg-laying hormone when they fire in prolonged afterdischarges of synchronous action potentials. Conventional intracellular recording from pairs of bag cells in A. dactylomela was used to demonstrate directly that electrical coupling underlies bag cell synchrony. Depolarizing or hyperpolarizing current pulses passed through the electrometer bridge into one cell produced electrotonic potentials of reduced amplitude and extended time course in the other. Coupling ratios for 63 pairs of cells ranged from 0.0004 to 0.087 with a mean of 0.057. At frequencies of 1 Hz, stimulating pulses of 100 msec duration produced summated responses in coupled follower cells that could be twice the amplitude of single responses. A single cell was often coupled to more than one other cell and in one case to five cells. Cells in opposite clusters were found to be weakly coupled. Extracellular recording indicated that the bag cell neurites in A. dactylomela do not extend as far along the pleurovisceral connective as they do in A. brasiliana and A. californica, suggesting that the coupling site is nearer the somata in A. dactylomela.

Simultaneous intra- and multiple site extracellular recordings also were used to examine bilateral interactions, synchrony and direction of propagation of bag cell spikes during spontaneous and evoked afterdischarges in A. brasiliana and A. dactylomela. Bag cell spikes were typically initiated on one connective near the terminations of the neurites and propagated toward the somata. This activity crossed the abdominal ganglion to the opposite cluster where impulses were usually initiated very near the somata and traveled outward toward the ends of the neurites. The sides on which this sequence of propagation was initiated could change many times during an afterdischarge. Tight synchrony across the abdominal ganglion was rare, however, because of backfirings, blockades and multiple sites of spike initiation. Prepotentials which potentiated to full spikes in one cluster could arise from improved invasion of action potentials from the contralateral cluster. Work supported by NIH awards NS 11255 and NS 08530 to J.T.H. and NS 70613 to J.E.B. and NSF grant PCM 76-18936.

552 EVIDENCE FOR CHOLINERGIC TRANSMISSION AT PHOTORECEPTOR SYNAPSES IN *HERMISSENDA*. Eliahu Heldman, Yoram Grossman*, Terry J. Crow and Daniel L. Alkon*. Section on Neural Systems, Laboratory of Biophysics, NINCDS, NIH, MBL, Woods Hole, MA 02543.

Each of the two simple eyes of the nudibranch mollusk *Hermissenda crassicornis* contains five photoreceptors (two Type A and three Type B, distinguished electrophysiologically). Each of the two optic ganglia contains thirteen second order visual cells. We attempted to identify neurotransmitters within this visual system. When the circumesophageal ganglia were incubated with labelled precursors for putative neurotransmitters and the products were extracted and analyzed by high voltage electrophoresis, four neurotransmitter candidates were shown to be synthesized and accumulated within the ganglia: acetylcholine (ACh), serotonin (5-HT), dopamine (DA) and histamine (HA). Direct enzyme assays suggested that the amount of neurotransmitter accumulated during 1-4 hours is directly proportional to the corresponding enzyme activity as measured in homogenates of the circumesophageal ganglia. When isolated eyes were incubated with labelled precursors for the above putative neurotransmitters only ACh was synthesized and accumulated at a detectable level (1-5 pmoles). Intense histochemical staining for acetylcholinesterase was observed in the region of the photoreceptor terminal branches. Histofluorescence localization of DA and 5-HT indicated that only one second order visual cell within each optic ganglion contained dopamine. The photoreceptors themselves did not fluoresce. Clear round vesicles were observed in endings of identified photoreceptors injected intracellularly with horseradish peroxidase. Intracellular recordings from identified photoreceptors were made while the nervous system was perfused with neurotransmitter agents: carbachol, DA, 5-HT, GABA, HA, and glycine. Only carbachol and HA had significant effects. Carbachol produced hyperpolarization of 5-15 mV accompanied by a drop of 10-60% in the input resistance. The reversal potentials for carbachol as calculated from the IV curve (-70 to -75 mV for Type A, and -85 to -90 for Type B photoreceptors) were similar to that of IPSP's which followed, one for one, neighboring photoreceptor impulses elicited either by light or intracellular current injection. HA produced hyperpolarization of 10-20 mV accompanied by a drop of 20-25% in the input resistance. Reversal potential for HA as estimated from IV curve was -90 to -100 mV. Eserine (10^{-3}M) reversibly eliminated or greatly reduced all photoreceptor interaction and increased membrane conductance 20-40%. The evidence presented here suggests that ACh serves as neurotransmitter for at least some of the photoreceptors in *Hermissenda*. Although HA was not synthesized by the photoreceptors, it may serve as a transmitter for other presynaptic neurons.

553 WEAK NEGATIVE COUPLING BETWEEN THE CIRCADIAN PACEMAKERS OF THE EYES OF APLYSIA. David J. Hudson* and Marvin E. Lickey. Dept. of Psychol., University of Oregon, Eugene, OR 97403.

The concept of a master biological clock controlling circadian rhythms is giving way to models involving multiple circadian pacemakers (PMs) which are coupled internally. The circadian system in Aplysia may be a case in point. There is a PM in each of the eyes and at least one more elsewhere. In the current experiments we asked whether the two ocular PMs are coupled. Forty-three Aplysia were released into very dim LL or DD. After 0 to 62 days both eyes were dissected and the phase of their rhythm was measured in DD by determining the time of the peak firing rate of CAPs in the optic nerve. Pairs of eyes removed after 0 to 7 days were always nearly synchronized; the time difference between the peaks of given pairs ($\delta\phi$) was between 0 and 1 h. Between 8 and 28 days, 77% of the pairs had $\delta\phi$ of 0 to 4 h and 15% had $\delta\phi$ of 10 to 12 h. After 29 to 62 days, 26% had $\delta\phi$ of 1.5 to 4.5 h and 61% had $\delta\phi$ of 9.5 to 12 h. In all 43 pairs only 14% had $\delta\phi$ between 4 and 8 h, none of which were in the range of 4.5 to 7 h, a span of potential phase angle differences which apparently is forbidden. Inhibitory neural connections are known to exist between the eyes, and it has been suggested that the eyes are neurosecretory. It is not farfetched, therefore, that the eyes might behave as PMs with weak negative internal coupling. This hypothesis predicts that after a long time in LL the majority of pairs of eyes should be in exact antiphase. Our data show, however, that in the majority of pairs the modal $\delta\phi$ is only 11.5 h, and occasionally as little as 9.5 h. Entrainment theory may explain even this small discrepancy. When we dissected a pair of eyes and prepared it for recording, we exposed it to a brief pulse of light. If the eyes were in exact antiphase, one of them would have been in its subjective day and the other in its subjective night. A generalized phase response curve (PRC) would predict that the light pulse should evoke a larger phase shift in the eye dissected in its subjective night than in the other eye. This asymmetric action would reduce the observed $\delta\phi$ of the pair from 12 h to some lesser value which is dependent on the amplitude and shape of the specific PRC for the light pulses of dissection. We therefore measured the PRC for dissection and found that its amplitude was about $\pm$ 2 h during the middle of the subjective night decreasing to nearly zero at the middle of the subjective day. This PRC goes a long way toward explaining the departure from 12 h shown by the pairs of eyes tested after long exposure to LL. A similar argument can be made for those pairs of eyes in which $\delta\phi$ departed slightly from zero. We believe that this is the first physiological demonstration of internal weak coupling between circadian PMs in a nervous system. NS 12374

554 PEDAL AND PARAPODIAL MOVEMENTS ELICITED BY CEREBRAL GANGLION NEURONS IN APLYSIA. Behrus Jahan-Parwar and Steven M. Fredman. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Locomotion in Aplysia involves coordinated rhythmic activity in both the foot and parapodia. As an initial step in analyzing the neural mechanisms underlying this activity we have examined several pathways that elicit reflex contractions of both organs and the role of several groups of neurons in the peri-esophageal ganglia. We have found that neurons in the identifiable A and B clusters of the cerebral ganglion mediate some pedal and parapodial movements. A semi-intact preparation was used. Aplysia were pinned rostrally and caudally and the foot longitudinally hemisected. Each parapodium was severed at its base with its connections to the pedal and pleural ganglia left intact, isolating it mechanically from the foot. Foot and parapodial movements were monitored via tension transducers.

The parapodia and foot both exhibited spontaneous rhythmic contractions. Those in the parapodia were abolished by cutting the pleuro-abdominal connectives; pedal contractions were not. This indicates that the rhythms are controlled independently. Reflex contractions of both organs could be obtained by touching the anterior tentacles or stretching either one hemi-foot or parapodium. Thus both direct and crossed reflexes are present. That all these reflex contractions could be obtained with the foot hemisected and the parapodia isolated suggests that a peripheral nerve net is not necessary for their spread or coordination and that control of the parapodia and foot resides in the CNS.

Intracellular stimulation of A and B cluster neurons caused both ipsilateral and bilateral pedal and parapodial contractions. Units recorded extracellularly from the parapodial nerves followed the driven somatic spike in the A neurons 1:1. Electrical stimulation of parapodial and pedal nerves caused antidromic spikes in both A and B neurons. Parapodial contractions evoked by A neurons persisted when synaptic transmission was blocked by adding Co^{++}. In addition, filling parapodial and pedal nerves with Co^{++} filled the somas of some A and B neurons. These results suggest that the A and B neurons are presumptive pedal and parapodial motor neurons.

The B neurons are active during reflexes elicited by tactile stimulation of the tentacles which suggests that they may help mediate them. Neither the A or B neurons fire significantly during proprioceptive reflexes. These are presumably mediated by neurons in the pedal and pleural ganglia.

This work was supported by PHS Grant NS 12483 to BJ-P.

555 CELLULAR LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN MOTOR-NEURONS FROM ASCARIS. Carl D. Johnson*, and Antony O.W. Stretton (Spon: P. Claude). Dept. of Zoology, Univ. of Wisconsin, Madison, WI 53706

We have begun to study the metabolism of putative neurotransmitters in the large parasitic nematode Ascaris lumbricoides by examining those motorneurons in the ventral nerve cord which send isolated circumferential commissures to the dorsal nerve cord. These include five of the seven different types of somatic motorneurons. Three of these neurons are excitatory to muscle in the dorsal half of the body, one is inhibitory to dorsal musculature and the fifth is inhibitory to ventral musculature (see Abstract by Walrond, Kass, Donmoyer, Moses and Stretton).

In order to isolate single commissures, the muscles and other internal organs are detached from the cuticle and hypodermis by treatment with bacterial collagenase. Processes of single motorneurons are then dissected as transverse strips of hypodermal tissue containing motorneuron commissures. These strips are incubated with [H^3]-choline and cold acetylCoA. Compared to hypodermal strips containing no commissure, elevated levels of acetyl-[H^3]-choline (ACh) are synthesized by strips containing a commissure of any of the three excitatory motorneuron types. Strips containing only commissures of inhibitory motorneurons do not synthesize elevated levels of ACh. This suggests that all of the excitatory motorneurons to the dorsal musculature are cholinergic and that the inhibitory motorneurons do not use ACh. (Supported by NSF Grant BNS 76-09641 and a Postdoctoral Fellowship from the Muscular Dystrophy Association)

556 A CHORDOTONAL ORGAN-LIKE STRUCTURE IN THE UROPODS OF CRAYFISH. Stanley R. Johnson* and Richard L. Roth* (SPON: Marion E. Smith). Dept. Biol. Sci., Stanford University, Stanford, CA. 94305.

In the rostromedial quadrant of both the exopodite and endopodite of the crayfish uropod there are patches of cuticular hairs which are longer and more densely packed than are hairs known to be sensitive to near-field water disturbances. These hair patches are overlapped by adjacent members of the tail fan so that they are protected from ordinary perturbations of the environment, but are sheared by promotion and remotion of the uropods. Consequently, neurons innervating these hairs have been assumed to signal postural changes of the tail fan, and ascending interneurons have been found which are especially sensitive to deformation of the patch of hairs on the endopodite (R. Fricke, personal communication).

We have recently discovered a chordotonal organ-like structure within the protopodite of the uropod of Procambarus clarkii which may reasonably be expected to supplement or complement the postural information supplied by the hair patches. This structure consists of a strand of connective tissue which inserts medially in the hard cuticle of the proximal end of the endopodite and laterally in the soft cuticle of the articulation between the endopodite and exopodite. A small branch of the 3rd root of the 6th abdominal ganglion enters the connective tissue strand at about one-third of the distance from the medial to the lateral insertion. Thus, the whole structure is Y-shaped, the base of the "Y" being the lateral insertion of the connective tissue strand. The structure, as a whole, contains about 26 neuronal somata of which the 10 largest (20-30 X 35-50 micrometers) lie near the site of nerve entry. The smaller cells (about 8 X 15 micrometers) lie, for the most part, towards either end of the connective tissue strand. Cobalt outfills of the 3rd root routinely fill most or all of the large cells, but few of the smaller cells.

We are beginning a physiological assessment of this presumed chordotonal organ and are engaged in selective labeling of its constituent cells and of other components of the 3rd root in order to establish whether there is an anatomically discernible segregation of different sorts of afferents within the ganglionic neuropil.

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557 CYCLIC AMP IN APLYSIA GILL: INCREASES BY PUTATIVE NEUROTRANSMITTERS. Procerfina R. Kebabian*, John W. Kebabian*, John W. Swann* and David O. Carpenter. Armed Forces Radiobiology Research Institute and National Institutes of Health, Bethesda, Maryland 20014.

Dopamine (DA) is found in substantial amounts in the gill of the marine mollusc Aplysia. When perfused, it induces contractions and also greatly facilitates transmission from identified motor neurons, some of which are clearly not dopaminergic (Neurosci. Abst. 2:508, 1976). Because of the possibility that at least the modulation of neuromuscular transmission might be mediated through cyclic adenosine monophosphate (cAMP), we have determined the ability of several putative neurotransmitters to affect cAMP levels in gill tissue.

Slices of gill tissue were incubated in seawater (30°C) for 20 min. The amines were added immediately prior to addition of the tissue to the seawater. At the end of the incubation period, the tissue was rapidly removed and frozen on dry ice. Subsequently, the tissue was homogenized; cyclic AMP was measured with the method of Brown et al. (Adv. Cyclic Nucleo. Res. 2:25, 1972).

Both DA (30 µM) and serotonin (5-HT) (100 µM) caused increases in cAMP levels to values 10 times control. Carbachol (100 µM) showed no significant effect. The half-maximal concentration of DA for this effect was 10 µM. While the 5-HT stimulation was immediate and approached maximal within 5 min, the stimulation by DA was slower and approached maximal only at about 20 min. Neither the DA- nor 5-HT-induced increases in cAMP levels were decreased when the experiment was performed in the presence of elevated Mg^{++} concentrations (200 mM), which is known to depress synaptic transmission. These results suggest that both transmitters are acting directly and not through activation of some intermediate receptor. In addition, the maximally stimulating effects of DA and 5-HT were additive, indicating the presence of two independent receptor types.

The increases in cAMP induced by both DA and 5-HT was blocked by both fluphenazine and cis-fluphenthixol. These drugs were essentially equipotent and gave nearly 100% inhibition at 100 µM. While neither fluphenazine nor cis-fluphenthixol had effects on resting cAMP levels, trans-fluphenthixol was unexpectedly found to stimulate basal cAMP levels and not block the action of either DA or 5-HT. The three ergot drugs tested--ergonovine, ergotamine, and lergotrile--did antagonize the effects of DA but, unlike their action in mammalian systems, they caused an accumulation of cAMP in absence of either amine. Although the site of the receptors has not been identified, our observations are not inconsistent with the hypothesis that DA may cause modulation of neuromuscular transmission by a cyclic nucleotide mediated modulation of contractility of smooth muscle fibers.

558 AN INTERNEURON IN DROSOPHILA SYNAPSES WITHIN A PERIPHERAL NERVE ONTO THE DORSAL LONGITUDINAL MUSCLE MOTOR NEURONS. David G. King. Yale University, New Haven, CT 06520.

Flight in flies is powered by a set of indirect flight muscles, including the dorsal longitudinal muscles (DLM). Activity in a pair of giant neurons descending from the brain reaches the indirect flight muscles and the jump muscle (TDT), apparently triggering an escape response to initiate flight (King, Neuroscience Abstracts 2:628; Tanouye, Neuroscience Abstracts 3).

Each giant neuron activates the DLM motor neurons through an interneuron (incorrectly labelled a motor neuron in last year's abstract, King, NA 2:628). After contacting the giant neuron this interneuron crosses the midline and exits the ganglion through the posterior dorsal mesothoracic nerve (PDMN), whereupon it synapses onto the five DLM motor axons.

The synaptic region is anatomically quite simple and is easily located in histological sections. In the proximal segment of the PDMN, within 50µm of the ganglion, are found nine large axons and numerous small axons. Three of the large axons (the TDT motor axon and its two accessory axons) are isolated from the remainder of the PDMN and soon separate laterally to innervate the TDT. The other six large axons consist of the five DLM motor axons surrounding the peripheral interneuron. Each of these six axons is typically a simple cylindrical process; synapses are formed by direct contact of the axons rather than by collateral branches or spines. Reciprocal synapses from the DLM axons onto the peripheral interneuron are occasionally observed; their function is obscure.

Distal to these peripheral synapses the interneuron terminates and the DLM axons increase in diameter. Thus the interneuron appears to be specialized for rapid conduction from the giant neuron into the periphery, bypassing the dendritic tree of the DLM neurons. Synapses onto the DLM axons occur at a region of relatively high input resistance (small diameter); the DLM axons then increase in diameter for rapid conduction to the muscles.

These peripheral synapses may prove useful in developmental or genetic studies of synaptic connectivity in which identification of specific contacts between identified neurons will be necessary. Thorough physiological analysis of this system should also be possible since the same synaptic contacts have been observed in other larger Dipterans (Valentino, Neuroscience Abstracts 3).

(Supported by USPHS grants NS07314 and NS05198.)

559 SYNAPTIC MODULATION OF ENDOGENOUS ACTIVITY IN WHITE CELLS OF THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. W. Michael King*, Michael F. Murphy*, and Norman R. Kreisman. (Spon: L.T. Happel) Depts. of Physiology and Pharmacology, Tulane University School of Medicine, New Orleans, La. 70112.

It has been reported that the white cells of the abdominal ganglion of Aplysia Californica receive weak inhibitory synaptic input resulting from stimulation of ganglionic nerves and connectives but no spontaneous synaptic input has been observed to date. The spontaneous activity of these cells is characterized by a regular spike output which is controlled by an endogenous pacemaker mechanism. This beating pattern often shows periods of waxing and waning of frequency and also periods when spiking ceases. In the course of other work in this laboratory, intracellular recordings have been made from the rostral and caudal white cells (R3-R15). Simultaneous recordings from two white cells at a time revealed that periods of cessation of activity were coincident. This cessation of activity appeared to result from a small hyperpolarization of 1-4 mV in amplitude which lasted up to several min. In some cases, the hyperpolarizations were preceded by a shorter duration 1 mV depolarization. When spiking resumed, it was at a lower frequency than normal and gradually returned to its previous rate over a period of minutes.

One explanation for the coincident periods of cessation of spike activity could be the presence of an inhibitory synaptic input from a common source. In order to test this possibility, simultaneous recordings were made from two rostral white cells until several coincident periods of spike inhibition were observed. The electrode from one white cell was then withdrawn and inserted into cell L11, which receives input from interneurons I and II. It was found that the periods of inhibition in the remaining rostral white cell were always coincident with a burst of action potentials in L11 which is characteristic of the action of interneuron II. In other experiments, simultaneous recordings were made from R14 and R15. R15 receives a biphasic postsynaptic potential (ILD-E) from interneuron II. Inhibitory periods in cell R14 were always coincident with ILD-E in R15, but not every ILD-E in R15 was coincident with inhibition in R14. These results indicate that the white cells R3-R14 receive synaptic input from interneuron II which can have both short term and long term modulatory effects on the firing rate of these cells, including complete cessation of spike activity. Stimulation of the right connective and nerves produces effects similar to those attributed to interneuron II in R15 as well as in the white cells. It is therefore possible that in each case the effects of nerve or connective stimulation are the result of excitation of interneuron II. (Supported by NIH grant NS 12419)

560 ANALYSIS OF THE FLIGHT PATTERN OF THE DORSAL LONGITUDINAL FLIGHT MUSCLE IN DROSOPHILA. J. H. Koenig* and Kazuo Ikeda. City of Hope National Medical Center, Duarte, CA 91010.

Simultaneous intracellular recordings were made from all 6 ipsilateral dorsal longitudinal flight muscle fibers (numbered 1 to 6, ventral to dorsal) in Drosophila while the animal was in stationary flight. The salient characteristic of the pattern generated by these 6 muscles is that they tend to fire at about the same frequency but at different times, with the exception of #5 and #6, which fire synchronously. An interval correlation analysis of this pattern reveals that the mechanisms involved in spacing of the firing of these muscles includes a combination of both "excitation" (so that a muscle fires earlier than it normally would have fired) and "inhibition" (so that a muscle fires later than it normally would), depending on which 2 muscles are involved.

Specific pairs of these muscles have stronger and more stereotyped interactions than other pairs and also show a higher frequency correlation. Thus, muscle pairs #1-2 and #3-4 exhibit the strongest interactions, which cause them to fire either in antiphase to each other or occasionally almost synchronously (within 1 msec). To effect synchronous firing, it can be seen that #1 fires earlier than it normally would have fired so that it fires within 1 msec of when #2 fires. No. 2's firing time appears unchanged. To maintain antiphase firing, it can be shown that #1's firing interval is shortened by about 30% while #2's concurrent interval remains the same or is lengthened by a variable amount. These interactions only occur when #1 and #2 would have fired within a relatively short interval of each other. This interval is equivalent to the "exclusion bands" in the phase histograms which have been reported by Wyman (1969). In addition, #1 usually fires slightly faster than #2. The same relationships are seen between #3 and #4, with #3 being equivalent to #1 and #4 equivalent to #2. Weaker but similar interactions are also observed between any other pair of muscles #1 through #4; but in this case, either muscle of the pair may exhibit a shortened or lengthened interval. Interactions of #1-4 with #5 and #6 are quite weak and difficult to define.

Underlying these spacing interactions, a common input is observed to drive all 6 dorsal longitudinal muscle fibers. (Supported by USPHS NIH grant NS 07442)

561 NEURAL CONTROL OF CIRCADIAN BEHAVIOR IN LIMAX MAXIMUS: ABSENCE OF AN ENDOGENOUS RHYTHM IN ISOLATED BRAINS. Stephen N. Kogge and Phillip G. Sokolove. Dept. of Biol. Sci., U. of Md. Baltimore County, Baltimore, MD 21228.

In experiments with intact giant garden slugs (Limax maximus), 24 hour temperature cycles ($15^{o}/10^{o}/15^{o}$C) were capable of entraining a locomotory circadian rhythm. The free-running rhythm was always observed to persist after the temperature cycle was discontinued (T constant = 15^{o}C). Similar experiments conducted on isolated brains showed no evidence of similar persistance of rhythmicity in pedal nerve output.

Formulation of a tissue culture medium matching the ionic and osmotic properties of slug blood allowed the continuous recording with suction electrodes from nerve trunks of isolated Limax maximus brains for periods of up to two weeks.

Brains held at constant temperatures, in closed and dark containers, showed no inherent rhythmicity of neuronal activity. When we alternately cycled the bath temperature from 10^{o}C to 15^{o}C and back (12 hours at each temperature), strong periodic responses developed by the end of 2-3 cycles. Typically, pedal nerve activity, measured as numbers of action potentials per two hours, increased during the high temperature phase and fell during the low temperature phase. In some cases non-pedal nerves showed the opposite response: neural activity clearly increased during the low temperature phase and decreased during the high temperature phase. When the temperature was held constant for 72 hours or more following at least 3 temperature cycles, no obvious rhythmicity in spontaneous activity was found in any trunk. Resumption of the temperature cycle always resulted in the same periodic output as has been seen earlier.

Two features of the in vitro neural activity are inconsistent with the circadian behavior of intact slugs: (1) activity in pedal nerves is normally greatest during the high phase of a temperature cycle whereas locomotor activity is greatest during the low phase; (2) no evidence of endogenous rhythmicity is found in vitro following cessation of a temperature cycle whereas the locomotor rhythm clearly persists. It is therefore unlikely that the Limax locomotor circadian rhythm is controlled by an endogenous neuronal oscillator located in the CNS.

562 RESISTANCE OF A CRAYFISH SENSORY INTERNEURON TO HYPER-INNERVATION. Franklin B. Krasne and Sun-Hee Lee*. Dept. Psychol., UCLA, Los Angeles, CA 90024.

Interneuron A (hereafter A) of the crayfish abdominal nerve cord normally receives synaptic input from mechanoreceptor neurons distributed over the side of the tail fan ipsilateral to A's axon and unilateral dendrites. We have found that: (1) When the 5 roots carrying mechanoreceptor axons of one side of the tail fan into the last abdominal ganglion are cut and the central and peripheral pieces sutured together, regeneration and reinnervation of ipsilateral A occurs over 2-6 weeks. (2) If cut roots from the contralateral tail fan are instead sutured to the ipsilateral central stumps, reinnervation by these "foreign" afferents occurs equally expeditiously. (3) If cut roots from one side of the tail fan are tied to roots on the intact opposite side of the body, the cut axons grow into the last ganglion along the roots to which they have been tied but they do not form synapses on A, which already has a normal complement of inputs; moreover, the axons do not cross through the neuropile to reinnervate A of their own side. (4) If roots of the intact side from the preceding experiment are now cut (after 6 weeks), the contralateral roots, which had already grown into the ganglion, rapidly (starting in about 1 week) form functional connections with the A whose normal inputs are now degenerating. We conclude that synapse numbers remain constant on A despite availability of acceptable regenerating afferents.

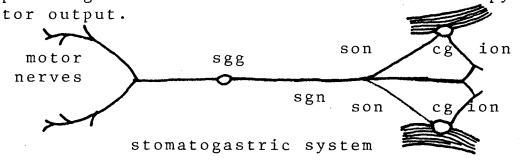
563 DIBUTYRYL CYCLIC AMP: PHARMACOLOGICAL MODULATOR OF THE AXONAL MEMBRANE SODIUM CHANNEL. Barry J. Kraynack, Major L. Cohn and Linda L. Kraynack*. Dept. Anesthesiology, Univ. Pgh. Sch. Med., Pittsburgh, PA 15261.

Local anesthetic agents produce nondepolarizing conduction blocks by inhibiting sodium ion flux through axonal sodium channels. The fact that pre and postsynaptic neural function is modulated by adenosine 3':5' monophosphate (cAMP) led us to examine in the present study whether at the axonal membrane level, the dibutyryl analog of cAMP (db cAMP) antagonizes blocking effects of agents which 1) bind specifically at or near the exterior opening of the sodium channel (tetrodotoxin 1 µg/.3 ml); 2) act strictly by a physicochemical mechanism (benzocaine 20 mg/ml, amobarbital 50 mg/ml); 3) act both through physicochemical means and at the axoplasmic receptor site of the sodium channel (procaine 15 mg/ml, chloroprocaine 20 mg/ml, lidocaine 20 mg/ml, mepivacaine 20 mg/ml, bupivacaine 2.5 mg/ml, tetracaine 10 mg/ml, naloxone 40 mg/ml, meperidine 25 mg/ml, ketamine 100 mg/ml). In groups of 12 naive male Sprague-Dawley rats (100 g) sciatic nerve blocks were produced by injecting .2 ml of the test drug around the sciatic nerve trunk at the junction of the biceps femoris and gluteus maximus. A frequency of 100% was obtained with all the local anesthetic agents tested; onset was very rapid (1-2 min). However, tetrodotoxin produced only a 60% frequency and a slow onset (10-15 min). Frequency and onset were unchanged by the addition of 2 mg of db cAMP to the test drug; however, db cAMP significantly shortened blocks induced by all the structurally unrelated drugs tested. Our findings agree with those of other investigators who reported that in isolated sciatic nerve preparations, conduction blocking effects of local anesthetic agents are antagonized by db cAMP. Moreover, we have found that db cAMP does not alter reabsorption of C^{14} lidocaine, administered in the subarachnoid space of cats and monkeys. Our study shows that the antianesthetic action of db cAMP is not contingent upon either the mechanism of action or the membrane sites where local anesthetic agents act. Therefore, we propose that db cAMP exerts its anti-anesthetic action by reestablishing the function of the axonal membrane sodium channel.

564 LOCALIZATION OF CATECHOLAMINES IN THE STOMATOGASTRIC NERVOUS SYSTEM OF THE CRAYFISH (PACIFASTACUS LENIUSCULUS T.). Pinky Drosten Kushner* (SPON: E.A. Maynard). Dept. of Biology, U. of Oregon, Eugene, Oregon 97403.

Dopamine appears to be present within the nerves and ganglia of the spiny lobster stomatogastric system (SGS) and may activate the pyloric motor output of the stomatogastric ganglion (SGG). To determine if dopamine is also present in the SGS of crayfish, isolated SGS nerves and ganglia were freeze-dried and heated in the presence of formaldehyde vapors. Whole mounts of the tissue displayed formaldehyde-specific fluorescence when viewed with UV light. As in the spiny lobster, fluorescence occurred in cell bodies of the two commissural ganglia (CG), in neuropil regions of the SGG, and in nerves connecting these ganglia. The fluorescence appeared catecholaminergic because of its green-yellow color, which did not fade rapidly when exposed to UV light, and because the areas of fluorescence were preferentially enhanced by pretreating the tissue with exogenous dopamine. As in lobster, fluorescent fibers projected from the CG to the SGG via the superior esophageal nerves (SONs) and the stomatogastric nerve (SGN). The crayfish ganglia were also linked by fluorescent fibers in the inferior esophageal nerves (IONs) and SGN, a pathway not found in the lobster.

Extracellular recordings of the crayfish SGS motor output showed that the pyloric rhythm was disrupted and slowed six-fold upon cutting the IONs; subsequent cutting of the SONs destroyed the residual rhythm. In contrast, lobster pyloric rhythm is essentially identical with and without the IONs but slows down several fold if the SONs are cut. It remains to be determined if the catecholaminergic fibers in the IONs in the crayfish are involved in the disruption of pyloric rhythm when the IONs are cut. These comparative observations are consistent with the hypothesis that dopaminergic neurons in the CG modulate SGG pyloric motor output.



565 INHIBITORY EFFECTS OF ESCAPE COMMANDS ON THE POSTURAL MOTOR SYSTEM OF THE CRAYFISH. John Y. Kuwada* and Jeffrey J. Wine. (SPON: Paul Lennard.) Dept. of Psych., Stanford U., Stanford, CA 94305.

The crayfish abdomen contains phasic and tonic motor systems. The phasic system is responsible for the initiation and coordination of the tail flip escape response. It is composed of a well-characterized neural circuit which includes command neurons. An action potential in a command neuron results in an escape response (Wiersma, J. Neurophysiol., 1947, 10, 23-28). The tonic system controls the postural activities of the abdomen. It contains two sets of antagonistic muscles, tonic extensors and flexors, and their associated motoneurons and command cells (Kennedy, Evoy, and Fields, Symp. Soc. Exp. Biol., 1966, 20, 75-109). One motoneuron of each set is a peripheral inhibitor which inhibits its respective muscle.

Previously, it was thought that the tonic and phasic systems were parallel but relatively separate and independent (Kennedy and Takeda, J. Exp. Biol., 1965, 43, 211-246). We have investigated interactions between those two motor systems in the isolated abdominal nervous system of the crayfish. Electrically-evoked escape commands in the phasic system inhibit ongoing activity of tonic flexor motoneurons centrally for up to several hundred milliseconds and drive the tonic flexor and extensor peripheral inhibitors with latencies of 5 to 10 milliseconds. In addition, electrical stimulation of sensory axons activates tonic flexor motoneurons but not the inhibitor. This activation is also inhibited by escape commands which immediately precede the sensory stimulation. This demonstrates that escape commands effectively prevent activation of the tonic system due to afferent inflow resulting from an escape response. Because of the slow time course of the tonic system, it is inferred that the role of this inhibition is to suppress postural behavior which would interfere with swimming often observed after an escape response. Furthermore, the response of the tonic system to sensory input exhibits rapid habituation upon repetitive stimulation of sensory axons. Another effect of the escape command initiated inhibition is to prevent such habituation. Thus, escape commands may preserve the excitability of postural reflexes as well as suppress incompatible behavior.

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566 SEPTATE JUNCTIONS AND ACTIVE NEUROMUSCULAR SYNAPSES IN EMBRYONIC LOBSTER HOMARUS AMERICANUS. Fred Lang and James J. Cole*. Department of Biology, Boston University Marine Program, MBL, Woods Hole, Massachusetts 02543.

Development of neuromuscular connections was studied in the abdominal muscles of embryonic lobsters using electrophysiological and ultrastructural techniques. Female lobsters extrude and fertilize several thousand eggs in August-September and brood them under the abdomen until the following May-June, when all embryos on a female hatch within 1-2 days. Development is essentially arrested when water temperature falls below 10° C (December-April).

Embryos were removed from females between December and April (eye index 350-400 µm), freed from the egg membrane and fixed for E.M. Myogenesis appears to follow the pattern of myoblasts fusing to form myotubes which then mature to myofibers. A growing neuron, characterized by a high density of granular vesicles, was observed to form a septate junction with a myoblast. Growing neurons were often observed in close proximity to myotubes. In this area, growth cones or "microspikes" are evident and the neuron is characterized by the presence of granular and dense cored vesicles, by "wavy" microtubules and by vesicular structures which may be continuous with intracellular tubules. These endings were only rarely observed near mature muscle fibers.

Excitatory and inhibitory neuromuscular junctions were observed on maturing and fully developed myofibers. These were often indistinguishable from adult synapses. Axoaxonal synapses have not yet been observed.

Recording from the abdominal muscles revealed resting membrane potentials of 50-80 mV. In 64% (91 penetrations in 13 preparations) of the penetrations, spontaneous miniature excitatory postsynaptic potentials (EPSP's) of up to 1.5 mV were observed. Large EPSP's (5-30 MV) could be evoked by tactile or electrical stimulation to the abdomen. The former stimulus would often elicit several tail flips in succession demonstrating that the circuitry for this reflex is present early in development.

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567 AN ELECTROPHYSIOLOGICAL AND ANATOMICAL INVESTIGATION OF THE GENITAL GANGLION IN APLYSIA CALIFORNICA. F.J. Lebeda and J.E. Blankenship. Marine Biomedical Inst. and Dept. Physiol. and Biophysics, Univ. Tex. Med. Br., Galveston, TX 77550.

The genital ganglion is located caudal to the abdominal ganglion along the genital nerve and contains approximately 20-30 neurons in the major cluster. Most of these cells can be backfilled with cobalt chloride via axonal iontophoresis of the caudal region of the genital nerve. Very few neurons, however, can be filled via the rostral portion of the genital nerve. Neurons are also found in one or more minor clusters or as individual cells scattered along the genital nerve. Nerve branches can be observed arising from the clusters toward the large and small hermaphroditic ducts and the receptaculum seminis.

Electrophysiological properties of the genital ganglion neurons have been examined using conventional stimulating and recording techniques. Intracellular recordings reveal that the neurons in the major cluster have a large resting membrane potential (-55.2 ± 1.1 (SEM) mV, n=43) and a high input resistance (19.4 ± 1.7 MΩ, n=24) which is constant over a wide range of hyperpolarizing potentials (Musgrave, unpubl. obs.). With sufficiently large hyperpolarizations, an after-potential is observed whose amplitude and polarity is voltage-dependent (Connor and Stevens, J. Physiol. 213: 31, 1971). Typically these neurons are silent at rest and have little synaptic input. In addition the cells are electrically coupled. Direct intracellular stimulation produces overshooting action potentials. Increasing the duration of the stimulus pulse causes repetitive firing whereupon the cell undergoes a long lasting period of accommodation. Most of these electrical properties are qualitatively similar to those of the L14 group in the abdominal ganglion (Kandel, Cellular Basis of Behavior, Freeman, 1976).

Stimulation of the caudal genital nerve elicits potentials 20-30 mV in amplitude (A-spikes) in the soma. Rostral genital nerve stimulation can often produce biphasic postsynaptic potentials comprised of an early, fast excitatory potential followed by a slow inhibitory response; both phases appear to increase membrane ionic conductance. With repetitive stimulation (5-10 Hz) several action potentials can initially be recorded intracellularly. Thereafter EPSPs and irregularly occurring action potentials may be observed. It is possible that both the accommodative property of the postsynaptic membrane and the slow inhibitory potential may play a role in the early cessation of the regenerative activity that occurs during repetitive stimulation. [Supported by NIH grant NS 11255, award NS 70613 to JEB and award NS 08531 to FJL and NSF grant PCM 76-18936.]

568 CRITERIA FOR CELL IDENTIFICATION: APPLICATION TO CENTRAL NEURONS OF PLEUROBRANCHAEA. Richard M. Lee and Reinhard Palovcik.* Dept. Neurol. and Behav. Sci., Edsel Ford Institute, Detroit, MI 48202.

Certain neurons of invertebrate species can be easily identified because they have unusual, externally observable properties, such as large size, atypical coloration or shape. In order to identify other cells, which do not have such unusual features, we have established methods which allow objective identification with an estimate of confidence.

We assume that in a large invertebrate ganglion, certain cells are unique and identifiable and possibly others are indistinguishable from one another. The basis for our identification system is the development of a fixed list of properties and a method which can be used for comparing cells. We define an "identified cell" as one for which average values can be specified for its properties, the variability in these properties can be specified, and it is distinguishable from other cells in the ganglion. In order to apply this definition, certain mathematical formulations must be developed, such as an "index of cell similarity." Such an index can be based upon distributions for parameters so that the probability that the sets of parameters for two cells are not accountable by chance variation can be computed.

In practice, a number of cells are penetrated in each of several preparations. For small ganglia, a particular cell found in the first prep. is said to be unique and identifiable if there exists one and only one very similar cell in each of the other preps. For large ganglia, where sampling methods must be employed, the following formula can be used for determining the number of preps required for a given level of confidence:

$$\frac{\text{LOG } (1-C)}{\text{LOG } (1-(SP/G)^2)}$$

where C is the confidence level, S is the number of cells studied per prep, G is the size of the population of cells sampled from, and P is the probability that the cell is in that group. SP/G may also be determined empirically by noting the number of occurrences of the cell in question for a given number of preps.

These methods were tested using a group of left ventral cells of the cerebropleural ganglion of Pleurobranchaea. An average of eight cells were penetrated in each of 11 preps. Parameters included soma size, location, synaptic input, spike shape measures, axons in brain roots, etc. -- a total of 40. Several variations of the procedures described above were evaluated. The resulting method led to the classification of three cells as "identifiable" with a confidence level of more than 95%.

569 NATURE OF INHIBITION ONTO GIANT INTERNEURONS IN THE CRICKET ACHETA DOMESTICUS. R.B. Levine, S.G. Matsumoto, R.K. Murphey. Dept. Biol. Sci., SUNYA, Albany, NY 12222.

Deafferentation during post-embryonic development in the cercal afferent to giant interneuron system of the cricket Acheta domesticus causes an increased effectiveness of inhibition onto giant interneurons (Murphey, Matsumoto, Mendenhal, 1976). This same increase also occurs when interneurons are non-surgically deprived of presynaptic activity (Matsumoto & Murphey, 1977). We are, therefore, currently studying the nature of cercal mediated inhibition in normal animals.

Giant interneurons in this system range from those which are not inhibited by cercal stimulation, to those receiving almost exclusively inhibitory input from the cerci. The lateral giant interneuron (LGI) receives excitatory input from cercal afferents ipsilateral to its axon and unilateral dendritic field. There is no sign of cercal inhibition onto this cell. Two giant interneurons with bilateral dendritic fields, the medial giant interneuron (MGI) and neuron 10-3, receive excitation from the cercus ipsilateral to their axons and inhibition from the contralateral cercus. Neuron 10-2, which is spontaneously active, receives mainly inhibition from both cerci. Thus its spontaneous activity is shut off upon stimulation of either cercus.

Intracellular recordings from the somata of 10-2 and MGI reveal a slow hyperpolarization in response to stimulation of the appropriate receptors. In the case of 10-2 this can be changed in size by a current intracellularly injected into the soma. Recordings from the dendrites of MGI reveal a burst of ipsps in response to stimulation of the contralateral cercus. These apparently unitary ipsps can also be changed in size by passing current. We conclude that the inhibition is post-synaptic.

Bath applied picrotoxin (10^{-6} to 10^{-5} M) causes MGI, which normally responds phasically to cercal stimulation, to become tonic. We are currently testing the effects of picrotoxin on the observed inhibitory potentials in the giant interneurons. Thus, the inhibitory pathway which is intimately involved in the plastic response of these neurons to various forms of deprivation is a chemical synapse impinging on the interneurons themselves and it is picrotoxin sensitive.

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570 INITIATION OF ESCAPE STREAMLINING IN THE CRAYFISH. Joseph Margiotta* and B. Walcott. Anat. Sci., Health Sciences Center, SUNY at Stony Brook, Stony Brook, N. Y. 11794.

Crayfish commonly escape from threatening stimuli by employing a sequence of rapid abdominal flexions and extensions. The rapid abdominal flexions provide propulsion. Motorneurons supplying abdominal flexor muscles are reliably excited by monosynaptic electrical input from either pair of medial (M.G.) or lateral giant (L.G.) interneurons. These giant interneurons integrate sensory information and are considered "decision fibers" crucial for the initiation of escape. During escape, the abdominal flexions are accompanied by postural adjustments of the thoracic appendages which may streamline the animal's body in its trajectory through the water. We have found that activity in the cheliped's coxopodite promotor muscle (mPro) contributes to the streamlining of this appendage. Using electromyography, we observe that cheliped promotion and tail flexion are always initiated simultaneously when escape is evoked via the M.G. circuit. Wiersma (J. Neurophysiol. 10:23, 1947) found that stimulation of M.G. or L.G. produced abdominal flexion and thoracic appendage promotion. These observations suggest that the different, yet complementary, thoracic and abdominal motor outputs may be initiated by impulses in the same giant interneuron.

We have evidence that supports this hypothesis. Intracellular stimulation of an abdominal M.G. produces a single or compound, short latency EJP in every ipsilateral fiber of mPro tested. An upper limit of the delay at the central M.G.-cheliped promotor motorneuron synapse was calculated to be less than 0.5 mSec. This suggested electrical coupling between the cells, and a neural organization similar to that responsible for abdominal flexion.

Extracellular stimulation of M.G. recruits several short latency spikes in axons of a nerve rootlet which appears to supply only mPro. When this rootlet is backfilled with 0.5 M $CoCl_2$, the cell bodies and complete arborizations of two large motorneurons reliably stain. The large central processes of these cells abut the edge of the M.G. axon in the first thoracic ganglion, just as the giant flexor motorneurons do in forming electrical synapses in abdominal connectives (Mittenthal and Wine, Science 179:182, 1973). Intracellular recordings from these promotor motorneuron processes are now in progress to determine the synaptic mechanisms underlying their excitation by the M.G.

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571 CENTRAL NERVOUS SYSTEM CONTROL OF THE GAIN OF A COMBINED MUSCLE AND SENSORY ORGAN IN THE CRAYFISH CLAW. J.D. Marrelli* and D. Angaut-Petit*(SPON : W.H. Evoy). CNRS,INP.10, Marseille, France.

The myochordotonal organ (MCO) of the crayfish claw is a combined muscle and sensory organ. The sensory cells discharge to movement about the Merus-Carpus (MC) joint of the limb. Activity of the motoneuron to the accessory flexor (AF) muscle associated with the sensory cells produces activity in these cells in the absence of any joint movement and also alters the receptive field of these cells. The effect of single AF spikes occurring in a train of spikes on MCO discharge has been studied under conditions of different joint positions, velocity and levels of active tension in the receptor muscle. In general a single AF spike activated most of the sensory cells, both flexion sensitive and extension sensitive cells, both tonic and phasic. Flexion units responded at high frequency (> 200 hz.) and short latency (<25 ms.). Extension units responded at also a high frequency (200 hz.) but a longer latency (>75 ms.). The activity of extension sensitive MCO cells normally excites the AF motoneuron and the activity of the flexion sensitive cells inhibits the activity of the AF motoneuron. Thus the action of the AF spike is to first produce negative feedback on to itself through the MCO system followed by a positive feedback. This possibly unstable situation is under the influence of the level of the active tension in the AF muscle. Low levels of active tension in the AF muscle resulted in shorter latency and higher frequency discharges in the MCO sensory cells to an AF spike. As active tension increases, the influence, both positive and negative, of AF motoneuron upon itself declines, to be replaced by greater sensitivity of the MCO organ to MC joint movement. This greater sensitivity to MC joint movement is due to the increase in AF muscle stiffness (the coefficient of displacement dependent force), and viscosity (the coefficient of velocity dependent force) that occurs with AF activity. As a result of these changes in AF muscle the receptive fields of the sensory cells of the MCO are increased in their size and shifted towards the position of greater MC joint extension. At this new level of AF muscle tension additional increases of AF activity influence MCO output not by direct action but through changes in AF muscle properties which improve the transmission of the MC joint movement to the sensory cells.

Thus the activity of the MCO sensory cells is under direct control of the CNS via the AF motoneuron in the early period of the AF motoneuron activity. However the primary stimulus of the MCO sensory discharge rapidly becomes the movement about the MC joint. The significance of the positive feedback of AF motoneuron onto itself may lie in the need for a rapid rise in the AF muscle tension at the onset of AF activity. This possibility is currently under investigation.

572 DIFFERENTIAL SENSITIVITY OF CRICKET CENTRAL NEURONS TO SENSORY DEPRIVATION. S.G. Matsumoto. Dept. Biol. Sci., SUNYA, Albany, NY 12222.

The response properties of two large primary sensory interneurons in the abdominal nervous system of the cricket Acheta domesticus were examined following prolonged periods of sensory deprivation. In an earlier study Matsumoto and Murphey (J. Physiol. 1977, in press), demonstrated that the medial giant interneuron (MGI) was sensitive to sensory deprivation. The deprivation procedure involved immobilizing the mechanoreceptive sensory hairs with a thin layer of facial cleansing cream (Clinique Cleansing Cream). MGI normally receives powerful excitatory inputs from mechanoreceptive hairs located on its ipsilateral cercus, and inhibitory and weakly excitatory inputs from sensory receptors on its contralateral cercus. Therefore, blocking the sensory inputs from a single cercus silences the major excitatory inputs to the ipsilateral MGI but the contralateral inhibitory and excitatory inputs remain functional. Chronic unilateral deprivation depresses the responsiveness of the MGI ipsilateral to the treated cercus. This depression results from an enhancement of the untreated contralateral inhibitory inputs and a decrease in the efficacy of the deprived excitatory inputs.

The effects of deprivation on another primary sensory interneuron with a different pattern of cercal innervation has now been studied. I have determined that the lateral giant interneuron (LGI) receives sensory inputs only from its ipsilateral cercus. Therefore, immobilizing the mechanoreceptive hairs on a single cercus completely abolishes any response in the ipsilateral LGI. No contralateral inhibition has been detected.

Unilaterally deprived specimens were reared and both MGIs and LGIs were tested intracellularly for their sensitivity to sound stimuli using a standard tone pulse of 500 Hz at 80 db. In 14 specimens the deprived MGIs were significantly depressed compared to their homologs. The LGIs of these specimens were not affected by the deprivation procedure. The data is summarized below:

	No. of Action Potentials			
	Right (treated)		Left (control)	
	X ± SD		X ± SD (10 trials)	
MGI	0.28	0.09	3.52	1.37
LGI	1.12	0.86	1.22	0.82

The results suggest that the lack of competition from inhibitory inputs prevents any depression of LGI. Supported by NSF research grant #BNS7523454 awarded to R.K. Murphey.

573 DETERMINATION OF GABA IN APLYSIA GANGLIA AND INDIVIDUAL NEURONS. Marilyn W. McCaman* and Bruce N. Colby*, (Spon: J.K. Engelhardt). City of Hope National Medical Center, Duarte, CA 91010.

GABA was determined in ganglia and individual neurons of Aplysia californica as the dinitrophenyl ethyl ester using gas chromatography-mass spectrometry. It was identified by retention time and by the relative abundances of 3 major ion fragments. Quantitation was achieved using isotope dilution GCMS with GABA-d_2 as an internal standard. The sensitivity of the procedure was less than 1 pmole.

Levels of GABA in ganglia varied over a 5-fold range (0.30, 0.60, 0.61, 1.03 and 1.60 pmoles/µg protein for abdominal, pedal, pleural, cerebral and buccal, respectively). When various parts of the ganglia were analyzed, most of the GABA was found in the nerves and in the neuropil. Only a small fraction was found in cell soma.

Numerous individual, identified neurons were also analyzed. Most of these neurons contained no detectable GABA (less than 0.2 pmoles/µg protein). However, a few, including two bilaterally-represented neurons in the buccal ganglion, tentatively identified as B3, contained measurable amounts (0.5-1.5 pmoles/µg protein). The values reported here for ganglia and for identified neurons are considerably lower than those obtained using the GABAase procedure.

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574 HISTAMINE AS A MULTIACTION NEUROTRANSMITTER IN THE CNS OF *APLYSIA CALIFORNICA*. Richard E. McCaman and David G. McKenna*, Div. of Neurosci., City of Hope Medical Center, Duarte, CA 91010.

Previous studies from this laboratory revealed that histamine is present in measurable quantities in only certain specific neurons within the CNS of *Aplysia* (Weinreich et al, Brain Res., 84, 341, 1975). Further studies by Weinreich and Yu (J. Neurochem., 28, 361, 1977) have demonstrated that the histamine-containing neurons (HNs) are also unique in their content of a specific histidine decarboxylase. We will present electrophysiological evidence in support of the role of histamine as a neurotransmitter in the central nervous system of *Aplysia*.

The HNs have been found to form synaptic connections with several different follower neurons within the *Aplysia* cerebral ganglion. The monosynaptic nature of these connections have been assessed by a variety of criteria including a) the augmentation of the evoked post-synaptic potentials (PSPs) after injecting TEA in the HN; b) the persistence of the PSPs in artificial sea water containing high levels of Ca^{++} which blocks polysynaptic pathways; and c) the reversible graded reduction of the PSPs in artificial sea water containing elevated levels of Mg^{++}.

Each HN appears to form direct synaptic connections with at least nine different follower cells. The follower cells can be subclassified according to the PSPs elicited by the connections they receive from the HNs. There appear to be five distinct types of PSPs: IEPSPs, IIPSPs, EIPSPs, EEPSPs and EPSPs. Application of histamine (by iontophoresis or by pressure ejection techniques) to the post-synaptic cell mimics the synaptically-evoked response in the different types of post-synaptic cells tested thus far (i.e., those exhibiting IEPSPs, EPSPs and EIPSPs).

Thus, the neurotransmitter role of histamine is supported by: 1) the unique cellular chemistry of the presynaptic neurons (HNs); 2) the evidence for the monosynaptic nature of the connections between the HNs and their follower cells; and 3) the ability of applied histamine to mimic the synaptically-evoked PSPs of specific follower neurons.

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575 SYNAPTIC DIVERSITY OF A MOTOR AXON INNERVATING A HOMOGENOUS POPULATION OF LOBSTER MUSCLE FIBERS. Dennis E. Meiss and C. K. Govind. Scarborough College, University of Toronto, West Hill, Ontario, Canada M1C 1A4.

Properties of the synapses and muscle fibers of the singly innervated distal accessory flexor muscle were examined in the first and second walking legs of the lobster, Homarus americanus. The synapses differ in physiological properties such that low release, highly facilitating synapses occur on proximally located muscle fibers and high release, poorly facilitating synapses occur on distally located fibers. Synapses along a single muscle fiber are similar even though they may arise from more than one separate primary axon branch. Also, a single primary branch may form the full complement of synaptic types. Muscle fiber input resistances, R_{in}, were correlated with synaptic properties such that low output, highly facilitating synapses occurred on fibers with low R_{in} (mean 101.1 kΩ) and high output, poorly facilitating synapses occurred on fibers with higher R_{in} (mean 131.7 kΩ). All muscle fibers were similar for other membrane electrical properties including time constants and membrane capacitances. Regression analyses show that R_{in} has little or no influence on the size of the intracellularly recorded synaptic potentials or on their facilitation properties. Thus, differences in muscle membrane properties cannot account for the observed synaptic diversity. Instead diversity is based on differences in transmitter output recorded by focal analysis of single synaptic foci. Thus, proximal muscle fibers are innervated by low quantal output synapses ($\bar{m}$ = 0.16) and distal fibers by high quantal output synapses ($\bar{m}$ = 2.90). Preliminary ultrastructural analysis of the diverse synaptic terminals suggest that the high-output, poorly facilitating terminals are characterized by a large area of presynaptic dense bodies compared to low output, highly facilitating terminals with a smaller area of dense bodies. (Supported by the Muscular Dystrophy Association of Canada.)

576 DISRUPTION AND STABILITY IN THE COMPOUND EYES OF CAVE-DWELLING CRAYFISH. DeF. Mellon, Dept. of Biology, University of Virginia, Charlottesville, 22901.

Severe degenerative changes of a structural and functional nature characterize the visual system of Procambarus erythrops, a Floridian cave-dwelling crayfish. The compound eyes of P. erythrops are reduced in overall size compared to those of a related epigean (surface-dwelling) species, P. clarkii. The relative corneal area has been diminished by roughly 80%, and all traces of ommatidial faceting have been lost. Histological studies reveal a reduction in ommatidial number and structural complexity, including the absence of crystalline cones. Four remnant optic ganglia are present, but cell counts are down, neuropile is truncated, and no laminar organization remains. Electroretinigrams recorded from the cornea of P. erythrops eyes show response amplitudes of about two orders of magnitude smaller than those recorded from P. clarkii eyes in response to light stimuli of identical intensity. In contrast to these severe visual deficits, the oculomotor system apparently is structurally and functionally intact. Eye-withdrawal and retina-stabilizing reflexes can be evoked by appropriate non-visual inputs. All of the eye muscles and all identifiable oculomotor neurons in P. clarkii are also present in the cave species. Although the motor system of P. erythrops has a stable functional organization which is in contrast to the disrupted nature of the visual pathway, a pronounced size reduction of oculomotor axons and eye muscles has occurred, and this suggests that the efferent organization may also be responding to the selective pressures of the cave environment. Our present detailed electrophysiological and fine structural observations of neuromuscular organization in the cave crayfish eye may suggest mechanisms through which these matching cellular changes in axons and muscle fibers are evolving.
Supported by USPHS research grant NS 04989.

577 THE ROLE OF PERIPHERAL INHIBITION IN THE TIMING OF A PHASIC BEHAVIOR. Dyane C. Mistick and Jeffrey J. Wine, Dept. of Psych., Stanford U., Stanford, CA 94305.

We have been investigating neural mechanisms responsible for temporal coordination of a simple, stereotyped behavior: the crayfish tailflip. A tailflip, which consists of a rapid flexion and reextension of the abdomen, occurs in less than a tenth of a second and so requires precise timing of antagonistic muscles. We wanted to know how peripheral inhibition, whose function in this system was heretofore obscure, could help coordinate the response.

The phasic abdominal flexor muscles are innervated by at least two excitatory axons and one inhibitory axon. We recorded intracellularly in the soma of peripheral inhibitor of the flexors and in flexor muscle fibers to establish the following points: (1) The peripheral inhibitor is effective in blocking muscle spikes and reducing tension. (2) During escape behavior, which can be initiated by a single impulse in an identified central command cell, the peripheral inhibitor often fires a delayed burst of impulses. Both the delay and the formation of the inhibitor's burst are centrally programmed. (3) Delay is the result of a polysynaptic pathway from the escape command cell to the inhibitor; the pathway includes the flexor motoneurons and a set of corollary discharge interneurons. (4) All of the synapses in the pathway from the command cell to the inhibitor appear to operate electrically, yet the response of the peripheral inhibitor shows marked 'facilitation' to a pair of command cell impulses as a result of temporal summation and the consequent recruitment of additional interneurons. (5) The peripheral inhibitor also receives monosynaptic, chemical EPSPs from the muscle receptor organs. The muscle receptor organs are stretched during flexion; their accelerating input sums with central excitation to enhance the burst in FI.

Thus, the flexor muscles are first excited and then inhibited in rapid succession. The central and peripheral connections to the peripheral inhibitor cause varying amounts of peripheral inhibition to be recruited, so that the intensity of peripheral inhibition is appropriate to both the intensity of the initial motor discharge and to the speed of flexion and reextension. The consequence of this arrangement seems to be that a brief but powerful flexion is insured in spite of variations in the output of the motor circuits.

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578 PHYSIOLOGICAL AND MORPHOLOGICAL IDENTIFICATION OF PEDAL NEURONS IN THE PULMONATE SNAIL MELAMPUS. Stacia Moffett and Linda Kahan.* Dept. Zool., WSU, Pullman, WA 99164.

The primitive pulmonate snail Melampus bidentatus crawls by utilizing the columellar muscles in repetitive crawl-step movements. This behavior is coordinated within the central nervous system and is therefore accessible to analysis at the level of neuronal interactions. A first step towards this goal has been the identification of efferent units in extracellular recordings. A relatively complex motor pattern has been obtained from tethered, crawling snails. Twenty efferent units are distinguished on the basis of spike height, nerve distribution and onset and pattern of activity within the crawl-step cycle. Probable functions for many of these units have been determined by correlating their activity with the snail's behavior.

In order to find the position of these efferent units in the central nervous system, the prominent pigmented somata in the pedal ganglia have been visually mapped. There is no evidence of symmetrically positioned cells or cell clusters in the two ganglia. Cobalt chloride backfilling allowed the somata of neurons with axons in each pedal nerve to be identified on the map. The majority of backfilled cells are found in the ipsilateral pedal ganglion but some are in the contralateral ganglion and a few are in the cerebral, pleural and visceral ganglia.

The activity of cells identified by the backfilling technique is being recorded intracellularly in whole-animal preparations. Suction electrodes positioned on the appropriate nerves allow identification of one-for-one action potentials in the soma and extracellular recordings. Correlations between the activity of the penetrated neurons and the movements of the snail provide clues to the function of each cell. The neurons identified thus far are driven by bursts of excitatory or alternating excitatory and inhibitory synaptic inputs during locomotion. (Supported by NSF Research Grant #BNS 76-09706 and by the NIH Biomedical Research Grant to WSU).

579 WHITE NOISE ANALYSIS OF ELECTROTONIC JUNCTIONS IN LEECH GANGLIA. G. P. Moore, B. M. Frazer*, C. M. Lent, J. A. Boles*. Dept. BME, U.S.C., Los Angeles, CA 90007, and Dept. Biol. S.U.N.Y., Stony Brook, N.Y. 11794.

We have investigated two junctions in the leech nervous system, that between the paired Leydig cells and that between Retzius cells, using white noise current-injection techniques. In most experiments currents having a Gaussian amplitude distribution (rms value about 1 nA) and a flat spectrum were injected into one of the paired cells while the transmembrane potential response of the homologous cell was recorded. In some experiments the injected current had a zero mean, while in others a hyperpolarizing or depolarizing DC bias was added. Bandwidths of injected currents varied between 10 and 50 Hz. Input (current) and output (voltage) were treated as continuous variables and first- and second-order Wiener kernels (cross-correlations) were calculated from several hundred seconds of data in each run. The first-order kernels were used to estimate the pulse response of the junction, and Fourier transformed to estimate frequency response characteristics. These characterizations were then independently checked against the response to positive and negative pulses of current, and to sinusoidally modulated currents. Second-order kernels were calculated to provide a characterization of the rectifying properties of the junction. Our results show that both junctions act as low-pass filters, but only the Leydig cell junction has rectification which depends upon the amplitude and frequency of the injected current. Such junctional properties are not predictable by measurements using DC currents alone. (A grant from Mr. Y. Nishimoto in support of this work is gratefully acknowledged).

580 DEVELOPMENT OF AN IDENTIFIED NEURON: MORPHOLOGICAL EFFECTS OF BLOCKING PRESYNAPTIC ACTIVITY OR REMOVING PRESYNAPTIC NEURONS. Rodney K. Murphey. Dept. Biol. Sci., SUNYA, Albany, NY 12222.

The development of a large abdominal interneuron, the medial giant interneuron (MGI), in the CNS of the cricket Acheta domesticus was studied. Both partial deafferentation and altered afferent activity levels, produced early in development, are known to alter the response properties of the MGI. The morphological consequences of these procedures have been examined by intracellularly injecting the neurons with cobaltous acetate and intensifying sectioned material using the Timm's method.

When the pair of homologous MGI's were injected in control specimens the neurons were seen to be very similar to one another. Three types of processes were found to project from the main dendrites. "Dendritic collaterals" which are cylindrical and may themselves branch. "Club Collaterals" which are approximately spherical and are mounted on a stalk do not branch. "Spines" which are similar to those found on vertebrate neurons and are distributed on all other types of dendritic process. The dendritic collaterals, club collaterals, and spines, while not identical, are similar in length and number on the bilaterally homologous neurons.

The paired MGIs in a specimen unilaterally deafferented at hatching are quite distinct from one another. The main dendrites of the deafferented neuron are shorter than controls, as reported earlier (Murphey et al., 1975, J. Comp. Neurol. 159:407). A dramatic alteration which could not be detected in the previous work, was a reduction in the length and number of dendritic collaterals and a reduction in the number of spines. Dendrites of the same neuron which are not deafferented are spared and may be expanded relative to controls.

Blocking afferent activity throughout postembryonic development alters the adult response properties of MGI. However, the main dendrites of the two MGIs in unilaterally treated specimens are identical in length and diameter. At the time of writing no conclusive evidence is available regarding differences at the level of dendritic collaterals or spines. The data is presently being collected. Thus it appears that it is the presence of the presynaptic connections, not the activity on the presynaptic neurons, which determines the morphology of MGI.

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581 OPTOKINETIC TRACKING OF OSCILLATING DRUMS BY THE CRAYFISH, *PROCAMBARUS CLARKII*. Richard F. Olivo. Dept. Biol. Sci., Smith College, Northampton, MA 01063.

Optokinetic tracking experiments are usually performed using a continuously rotating drum, which a crayfish or other crustacean tracks with slow eye movements that follow the drum and periodic rapid flickbacks that return the eyes to their starting positions. If a sinusoidally oscillating drum is used instead, the eyes under appropriate circumstances track the drum without flickbacks, and the amplitude of tracking can be used as a quantitative measure of the stimulus's effectiveness, as we have shown in color discrimination experiments (Neurosci. Abstr. 1:902, 1975).

In the present experiments, we further explored the response to oscillating drums by using a standard stimulus and systematically varying its parameters. The drum consisted of a high-contrast array of 20° opaque (black) stripes and bright translucent stripes that were lit from outside by a red source (650 nm, stripe luminance 5 ft.c.). Sinusoidal oscillations of 22 - 0.4 cyc/min (0.37 - 0.007 Hz) were imposed by an eccentric drive and variable speed motor; the standard oscillation amplitude was 36°. Eye movements were monitored by a capacitative transducer on the left eye.

Decreasing the oscillation frequency (in steps), starting at 22 cyc/min, produced an increase in the amplitude of tracking; tracking amplitude increased linearly as the log of the frequency decreased. At frequencies lower than 5 cyc/min, flickbacks appeared; the number of flickbacks per cycle increased as the oscillation frequency was made lower. Since the total excursion of the eye is limited by flickbacks, the tracking amplitude reached a maximum (about 20°) at low oscillation frequencies (<1 cyc/min). Decreasing the luminance of the stripes (with ND filters at the source) or reducing the horizontal extent of the stimulus (with screens placed between the animal and the drum) substantially depressed the tracking amplitude and the number of flickbacks per cycle, but did not shift the oscillation frequency at which flickbacks first appeared. Similarly, decreasing the drum's amplitude of oscillation depressed the flickback rate but did not shift the frequency at which flickbacks were initiated.

In these experiments, the animals were restrained and usually remained quiescent. Occasionally, animals initiated locomotory movements that were accompanied by large-amplitude movements of the eyes. The cessation of the spontaneous locomotory and eye movements revealed a tracking response of increased amplitude, which then declined to normal levels over a period of several minutes. These findings provide support for the hypothesis that the main function of the optokinetic system is to correct eye movements that are generated non-visually during locomotion.

582 A PRESSURE SYSTEM FOR INTRACELLULAR AND EXTRACELLULAR EJECTIONS OF PICOLITER VOLUMES. Joyce K. Ono*, Richard E. McCaman, and David G. McKenna*. (Spon: J.T. Holden). Div. of Neurosciences, City of Hope Medical Center, Duarte, CA 91010.

We are attempting to isolate and characterize neuroactive substances endogenous to neural tissues by using the responses of identified neurons in the CNS of a marine mollusc, Aplysia californica as a bioassay. We have developed a pressure ejection system which delivers picoliter volumes of test solutions to the soma and proximal axonal areas of various Aplysia neurons. This pressure ejection system utilizes short duration pulses (milliseconds) generated by conventional laboratory stimulators. A time marker whose polarity and amplitude are adjustable and whose duration is proportional to the ejection pulse duration is provided. Conventional micropipettes are utilized in this system and simultaneous intracellular recording from the pressure micropipettes is possible for intracellular injections. The amount of substances ejected can be directly quantified since the ejected volumes are linearly related to the adjustable parameters of pulse pressure and duration.

Comparisons of the responses of various neurons in Aplysia to acetylcholine delivered by iontophoresis and by the pressure ejection system demonstrate that the pressure system may be used in place of iontophoresis and has several advantages over iontophoresis: a) un-ionized substances may be ejected; b) reproducible responses are more readily obtained since problems of desensitization and the need for braking currents are circumvented; and c) the amount of substance ejected can be directly quantified. The pressure system has also been used for cobalt staining of individual Aplysia neurons to demonstrate its use in intracellular injection.

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583 SYNAPTIC MODULATION BY AN IDENTIFIED OCTOPAMINERGIC NEURON IN THE LOCUST. Michael O'Shea* and Peter Evans* (SPON: M. Burrows). Zool. Dept., Cambridge University, England.

Enzymic assays for octopamine were performed on a physiologically identified and isolated neuron in the third thoracic ganglion of the locust. It is unpaired, has a central cell body and projects via two axons bilaterally to the paired jumping muscles of the hind legs. These muscles also receive input from each of just three pairs of motoneurons: a fast and slow excitor and an inhibitor. The unpaired cell is not a motoneuron. Its soma and axons contain significant amounts of octopamine, the concentration in the axons being approximately four times that in the soma. The motoneurons do not contain octopamine.

We have shown that octopamine applied to the muscle in low concentration potentiates the force and facilitates the EJP produced by the slow excitatory motoneuron and, in contrast, that it reduces the amplitude of the inhibitory motoneuron's IJP. These and other effects can also be obtained by direct intracellular stimulation of the modulatory octopaminergic neuron. Neuromuscular transmission involving the fast motoneuron appears not to be affected.

Spikes in the unpaired cell can be elicited by natural stimulation in a variety of sensory modalities including visual, tactile and auditory. Left and right axons each have their own site of impulse initiation and although spikes are normally initiated together, they can arise independently in one or the other axon. By means of a single cell it is therefore theoretically possible to modulate motor activity independently in left and right members of a pair of muscles.

The ganglion contains several similar unpaired neurons which innervate other muscles. The transmitter has not yet in each case been identified although some contain octopamine and all are probably aminergic. Central connections made by these cells are the subject of current research and preliminary results suggest a modulatory function similar to that in the periphery.

This work was supported by the Science Research Council, the Agricultural Research Council and a Nuffield Research Grant awarded to Dr. M. Burrows.

584 LOAD COMPENSATION IN THE CRAYFISH ABDOMEN. Charles H. Page. Dept. Physiology, Rutgers University, Piscataway,N.J. 08854.

Application of a load to oppose postural extension of the crayfish abdomen evokes a load compensating increase in the discharge of the excitatory superficial extension motorneurons (SEMNs). Unit analysis of these SEMN responses during extensions generated by stimulation of command fibers indicates that most or all of the excitatory SEMNs increase their discharge in response to the load stimulus.

It has been suggested (Fields, J. Exp. Biol., 44, 455, 1966) that the load excites the tonic sensory neuron (SR1) of the muscle receptor organ which reflexly elicits a load compensating increase in the activity of one of the smaller SEMNs (#2). However, in the present experiments the SR1 was silent during most of the extensions against a load. In the few extensions where the SR1 was active, its activity contributed to the extension since it was proceeded by an increase in SEMN #2 discharge; these extensions were accompanied by increased discharge in the other excitatory SEMNs as well.

To test whether SR1 activity evoked the observed increases in SEMN discharge, the load was restricted to a single abdominal joint and the unit discharge of the SR1 and SEMNs from the joint were monitored. In 6 of the 7 preparations the SR1 was silent while the SEMNs increased their discharge when extending against a load.

It is concluded that the principle load sensors in the abdomen are not the MROs. The load compensating responses of the SEMNs must result from the activity of an unidentified load sensitive sensory system.

585 NEUROBIOLOGY OF HOMEOTIC MUTANTS IN DROSOPHILA. John Palka, Dept Zoology, Univ. Washington, Seattle, WA. 98195.

In the homeotic mutants of Drosophila, structures of a given segment are transformed into those of a different segment. I have studied the central projections of the peripherally located sensory cells of several mutants affecting the thorax: bithorax (bx, anterior meta- to anterior mesothorax); postbithorax (pbx, posterior meta- to posterior mesothorax; bx and pbx combined to produce flies with 4 wings (instead of 2 wings and 2 halteres) and 2 pairs of mesothoracic legs, plus a strong though incomplete transformation of the central regions of the thorax; and Contrabithorax (Cbx, meso- to metathorax), a mutant with variable expressivity which in extreme cases yields flies with 4 halteres and 2 pairs of metathoracic legs.

I studied the sensory projections of wings and halteres by backfilling the purely sensory wing and haltere nerves with cobalt. Each appendage has a characteristic distribution of fibers within its own segment, and also produces tracts which travel longitudinally, some as far as the head.

In bx flies (anterior wing/posterior haltere), a supernumerary anterior-directed tract is produced medial and ventral to the wild-type haltere tract which continues to be present. pbx flies (anterior haltere/posterior wing) have normal looking projections as would be expected from the fact that in the wing all the receptors are located in the anterior region and the mutation therefore introduces none of them into the metathorax.

In 4-winged flies (bx/pbx), a wing-like projection occurs in the metathorax as well as in its usual location in the mesothorax. However, many fibers which follow the course typical of the wild-type haltere are present, even though no haltere tissue can be identified on the fly's surface.

In 4-haltere flies (Cbx), the wing projection within the mesothorax is lost and fibers with at least some of the branching and staining characteristics of haltere axons are introduced.

The mutants thus show a mixture of appendage-specific and segment-specific projection patterns. Further analysis based on mosaics in which the cns is wild-type and the appendage mutant, or vice versa, is under way.

This work was initiated during the tenure of a Guggenheim grant with Dr. Peter Lawrence (MRC Labs of Molecular Biology, Cambridge, England) and is now supported by PHS grant NB07778.

586 PRESYNAPTIC INHIBITION OF DESCENDING MOVEMENT DETECTING NEURONS IN THE LOCUST. K.G.Pearson and C.S.Goodman*, Dept. of Physiology, Univ. of Alberta, Edmonton, Canada and Dept. of Zoology, Univ. of California, Berkeley, Ca. 94720.

The descending contralateral movement detector interneuron (DCMD) in the locust receives visual input in the brain and has an axon descending the contralateral connective to the thoracic ganglia. Intracellular recordings from the axon of this neuron in the meso- and metathoracic ganglia demonstrate the spontaneous occurrence of postsynaptic potentials (PSPs). These PSPs can be either hyperpolarizations or depolarizations at resting potential, but usually they are only observed as depolarizations after hyperpolarizing the axon with injected current. PSPs are evoked in the axon by stimulation of the contralateral connective, the cerci and sensory axons in the leg. PSPs follow 1:1 action potentials in the DCMD in the opposite connective. Intracellular injection of chloride ions increases the amplitude of the PSPs in the depolarizing direction, while chloride-free saline and picrotoxin abolishes them. Thus the generation of the PSPs appears to a conductance to chloride ions, the equilibrium potential is close to resting potential, and the putative transmitter is GABA. The conductance changes underlying these PSPs may either decrease transmitter release or block transmission through the terminal branches of the axon. Two observations indicating that presynaptic inhibition can occur are (1) in many animals there can be a prolonged or transient block of transmission across the monosynaptic junction from the DCMD to the fast extensor motoneuron (FETi), and (2) high frequency stimulation of the contralateral connective (which evokes PSPs in the DCMD terminal axon) can transiently block transmission from DCMD to FETi.

587 THE PHYSIOLOGY AND ANATOMY OF AN ARTHROPOD MUSCLE WITH POLYNEURAL INNERVATION. Christine E. Phillips, Dept. of Biology, University of Oregon, Eugene, OR 97403.

The well studied classical arthropod muscles are considered to have a simple frequency modulated neural control because they receive only one or two excitatory axons and one inhibitor. One group of muscles, the multiply innervated flexors of arthropod walking legs, prove to be an exception to this rule. Not only do they receive input from more motorneurons, they appear to be the effectors driven by a central pattern generator during walking, while their antagonists, the extensors, follow reflexively.

The metathoracic flexor tibiae of the locust was chosen for study. It has previously been shown to receive innervation from 7 excitatory motorneurons (3 fast, 2 intermediate, 2 slow) and 2 inhibitory neurons. An eighth excitatory flexor tibiae motorneuron (fast) has been found over the course of this study. The peripheral distribution of these neurons was not known. Flexor motorneuron somata were impaled, identified, and orthodromically stimulated while a second microelectrode was moved to monitor excitatory junction potentials in the muscle. No localization of endings was found in any region of the muscle, rather endings from each motorneuron were found throughout the muscle. These findings are in agreement with electron micrographs of axon profiles in the major nerve branches to the muscle, in which eight to ten axons are seen in each branch. Further, single muscle fibers receive at least three and as many as seven different post synaptic potentials. Thus, there is no apparent organization of motorneuron endings on the muscle.

The proximal and distal regions of the muscle are different, both in structure and function. When tension production is compared in the proximal and distal halves of the muscle, the fibers in the proximal half have faster rise times to peak tension (150 ms-prox., 350 ms-dist.) and fatigue more readily than those in the distal half. Ultrastructural differences are also present. Sarcomere lengths are shorter in the proximal region of the muscle (5.3 u-prox, 6.9 u-dist, $\pm$ 0.5 u). Actin to myosin ratios also vary from 2.4:1 in the proximal region to 5.3:1 in the distal region. Thus the flexor tibiae, in having one region for rapid contraction and another region for sustained tension production, is effectively a combination of the classical phasic and tonic muscle types. (This work was supported by PHS 5 T32 MH 14281-02 and NSF BMS75-00463).

588 STRUCTURE OF NEURONAL AND GLIAL MEMBRANES AT THE SQUID GIANT SYNAPSE. D. W. Pumplin and T. S. Reese. LNNS, NINCDS, NIH, Bethesda, MD 20014 and Marine Biol. Lab., Woods Hole, MA 02543.

The synapse between the 2nd order giant axon and the most distal of the 3rd order giant fibers in the stellate ganglion of the squid, L. pealei, was examined with the freeze-fracture technique. Stellate ganglia were excised and fixed in 2.5 percent glutaraldehyde in buffered 0.8 M sucrose. The postsynaptic axon was then injected with ruthenium red to aid in locating the "giant" synapse during freeze-fracturing. Examination of thin sections from comparable preparations confirmed previous reports (Young, Phil Trans Roy Soc B, 229:465; Hama, Z Zellforsch, 56:437) that a continuous glial and connective tissue sheath separates the pre- and postsynaptic axons, except where it is penetrated by thin processes (1-2 μm in diameter) of the postsynaptic axon. These axonal contacts were characterized by bands of fuzz, subjacent to each membrane and in the clefts between them, and by clusters of synaptic vesicles in the presynaptic axon. Further evidence of their synaptic nature was provided by stimulating the presynaptic axon at 20 Hz, while recording postsynaptically. This resulted in depletion of the synaptic vesicles near synaptic junctions. The structure of the membranes at these synaptic junctions was similar to that of many other types of excitatory synapses in that the distribution of particles on pre- and postsynaptic membranes was different. The presynaptic membrane had a disk-shaped aggregate of loosely clustered large particles (10 nm) approximately 0.3 μm in diameter. The postsynaptic membranes had coextensive aggregates of similar particles which, unlike the presynaptic ones, were on the external membrane leaflet; the concentration of particles in these aggregates approached 2000/μm^2. Thus, the postsynaptic membrane at this synapse resembles that at excitatory synapses in the mammalian brain but differs from excitatory cholinergic synapses on muscle and sympathetic ganglion cells. Two types of glial processes were readily distinguished from axonal membranes by the size, concentration and distribution of their membrane particles. Clusters of particles on the cytoplasmic leaflet of glial membranes in exact register with clusters of particle imprints on adjacent membranes were presumed to be components of intercellular junctions. These junctions closely resembled gap junctions in vertebrates but differed from those in arthropods where the constituent particles are on the external leaflet of the membrane. Thus, there may not be a distinct type of "invertebrate" gap junction.

589 NEURAL CONTROL OF THE PENIS RETRACTOR MUSCLE OF APLYSIA. M.K. Rock*, J.E. Blankenship, and F.J. Lebeda. Marine Biomedical Inst. and Dept. Physiol. and Biophysics, Univ. Tex. Med. Br., Galveston, TX 77550.

The excitatory innervation and physiology of the penis retractor muscle of A. californica have been examined using combinations of intracellular, extracellular and tension recording from neurons in the right pedal ganglion and from the muscle. The mean resting potential from 48 individual muscle fibers recorded with microelectrodes was -65 mV ($\pm$7 mV, SD). Simultaneous intracellular recording and stimulation from pairs of muscle fibers revealed that the fibers are electrically coupled. Almost all fibers have spontaneously occurring excitatory junctional potentials (ejps) of varying amplitude and time course.

Axonal iontophoresis of cobalt chloride applied to the cut end of the nerve innervating the retractor muscle has resulted in the backfilling of neurons in restricted regions of the right pedal ganglion. Impaled homologues of the neurons in one of these clusters were shown to be excitatory motor neurons innervating several muscle fibers directly with one-to-one constant latency ejps. Fibers receiving direct ejps also had other spontaneous ejps which could represent input produced by direct innervation by other motor neurons or electrotonic spread of ejps produced in coupled fibers. The innervation of the muscle is thus diffuse and polyneuronal. Directly produced ejps were seen to facilitate strongly to motor neuron stimulus frequencies of up to 10 Hz. Repetitive motor neuron activity elicited a contraction which was blocked when the muscle alone was perfused with sea water containing elevated magnesium concentrations; contractions could not be blocked when either the ganglion alone was perfused with elevated magnesium sea water or when the muscle alone was perfused with sea water containing elevated calcium concentrations. Motor neurons have a spontaneous irregular firing pattern and this, along with the innervation pattern and coupling among the fibers, accounts for the rich tonic excitatory input seen in this slow smooth molluscan muscle. Work supported by NIH grant NS 11255, award NS 70613 to JEB and award NS 08531 to FJL and NSF grant PCM 76-18936.

590 ACTIONS OF SEVERAL PUTATIVE NEUROTRANSMITTERS ON THE GILL OF APLYSIA. Peter C. Ruben*, John W. Swann* and David O. Carpenter (SPON: D. Evans). Armed Forces Rad.Res.Inst.,Bethesda, Md. 20014

Nerve-muscle interaction in the gill of Aplysia constitutes a highly suitable preparation for study of the presence of receptors for specific neurotransmitters. Biochemical assays have shown the presence of high concentrations of dopamine (DA) in the Aplysia gill. We have studied the effects of DA and other putative transmitters on spontaneous and induced contractions in the isolated gill pinnule of Aplysia californica in an effort to determine which of these act on the gill muscle and which may be the neuromuscular transmitters for identified gill motorneurons.

A pinnule was removed from the gill and suspended by a ligature through the efferent vessel and attached to a Grass tension transducer. The afferent vessel was tied to a fixed anchor in the bath. Contractions were amplified and recorded on a Brush recorder. Drugs were applied by bath perfusion in either normal seawater, high Mg^{++} (150mM) seawater, or 30mM $CoCl_2$ seawater to depress synaptic activity. Reproducible contractions were elicited by a speaker-driven tactile stimulator.

Our studies focused on three types of contractions: spontaneous pinnule movements, contractions induced by tactile stimulation, and those induced by the perfusion of DA at 10^{-4}M. High Mg^{++} seawater blocked spontaneous and stimulus-induced contractions, but had only a slight reducing effect on DA-induced contractions. Seawater containing $CoCl_2$ blocked spontaneous and stimulus-induced contractions and had little or no effect on DA-induced contractions. Contrary to expectations, neither acetylcholine nor glutamate caused contractions in this preparation. These substances induce contractions when perfused through the gill in an intact preparation including the PVG and the gill ganglion. GABA, aspartate, histamine, epinephrine and norepinephrine had neither excitatory nor inhibitory effects. Carbachol and DA were inhibitory on specific muscles in stimulus-induced contractions. Octopamine and phenylethanolamine were also inhibitory to spontaneous and stimulus-induced contractions. Serotonin, which is present in peripheral gill neurons (Peretz and Estes, J. Neurobiol. 5:3, 1974), was inhibitory on spontaneous and stimulus-induced contractions as well as on DA-induced contractions.

Our results suggest that there are excitatory DA receptors on some smooth muscle cells in the gill of Aplysia. In addition, these experiments confirm the functional role of a peripheral nerve net, which mediates spontaneous and stimulus-induced contractions. The dominant effect of several putative neurotransmitters on the peripheral nerve net is inhibitory.

591 THE EFFECT OF INTERNAL Ca^{++} CONCENTRATION IN PARAMECIUM. Youko Satow. Lab. of Molecular Biology, Univ. Wis., Madison, Wisconsin 53706.

The internal Ca^{++} concentration in Paramecium aurelia is changed with the ionophoretic injection of Ca^{++} or $EGTA^{--}$, bathed in a K solution (KCl 4 mM, $Ca(OH)_2$ 1 mM, citric acid 1 mM, Tris 1.3 mM, pH 7.2). Fifteen sec, 10^{-9} A injection of Ca^{++} through an electrode filled with 100 mM $CaCl_2$ reduces membrane resistance by 30%, hyperpolarizes the membrane. The cell recovers from these effects within a minute. Additional injection of Ca^{++} has little effect. The membrane resistance becomes 200 to 250% of initial value with over 60 sec, 10^{-9} A injection of $EGTA^{--}$ through an electrode filled with 100 mM K_2EGTA. The effect of $EGTA^{--}$ injection depolarizes the membrane for some minutes. $EGTA^{--}$ injection following TEA^{+} injection has little effect on the resting membrane resistance or the resting membrane potential. The current-voltage relation at hyperpolarizing half after $EGTA^{--}$ injection is similar to that after TEA^{+} injection. These results show that the internal Ca^{++} controls a K permeability in P. aurelia.

A prolonged depolarization after the action potential, which is known as a Ca spike, appears by $EGTA^{--}$ injection. The slope of the plateau potentials in various Ca^{++} concentrations in a TEA solution where TEA^{+} replaces the K^{+} in the K solution is close to the theoretical slope (29 mV for ten-fold change in $[Ca^{++}]_o$) and is parallel to the slope of the peak potential. Therefore, the prolonged depolarization is due primarily to a Ca current and appears after the inhibition of K permeability and the reduction of internal Ca^{++} concentration.

An effect on the maximal rate of rise of action potential is seen by the change of internal Ca^{++} concentration; i.e., the maximal rate of rise is smaller by Ca^{++} injection and larger by $EGTA^{--}$ injection in the K solution. The change of Ca current appears on the I-V curve at depolarizing half. One possible interpretation will be discussed in comparison with the I-V curve by TEA^{+} injection.

Membrane potentials are recorded with intracellular microelectrodes filled with 500 mM KCl and the relative change of the internal Ca^{++} concentration with the injected Ca^{++} or $EGTA^{--}$ is estimated by the change of the peak potential before and after injection of ions.

The work was supported by NSF BMS 75-10433 to C. Kung.

592 L9-INDUCED GILL CONTRACTIONS IN APLYSIA ANTAGONIZED BY THE DOPAMINE RECEPTOR BLOCKERS FLUPHENAZINE AND ERGOMETRINE. John W. Swann*, C. Nelson Sinback* and David O. Carpenter (SPON: M. L. Wiederhold). Armed Forces Rad. Res. Inst., Bethesda, Md. 20014.

The gill of Aplysia californica contains 3 µg dopamine (DA) per gram of tissue--a very high concentration. Our aim is to determine the physiological role of DA in the gill. Experiments were performed on the semi-intact gill preparation of Aplysia. The preparation consists of the abdominal ganglion and the gill. The ganglion was isolated from the rest of the preparation in a vaseline-sealed chamber. The gill was cannulated and perfused. As we have previously reported, perfusion of the gill with DA at threshold concentrations of 10^{-7} to 10^{-6} M results in highly reproducible contractions of efferent vessel trunklets, pinnule longitudinal muscles, and afferent vessel. These contractions in part mimic gill contractions due to the motor neuron L7. DA perfusion also dramatically modulates (i.e., enhances) the gill contractions of L7 and at least one other motor neuron, LDGl. Biochemical assay of the soma of L7 found very little DA (1 µM). This finding suggests that L7 may not be dopaminergic.

More recently, we have found that induced spiking of L9 neurons causes the same contractions as DA and L7. As many as 3 of these L9 cells and L7 have been recorded from in the same preparation. The largest of the L9 cells causes contractions of the whole gill (i.e., all efferent vessel trunklets, pinnule longitudinal muscles as well as the afferent vessel). Other L9 cells control the movement of these same muscle groups but in a more restricted area of the gill. For instance, in five preparations we have recorded from two smaller L9 cells, one of which induced contractions of the anterior half of the gill, while the other cell controlled the posterior half. Unlike L7, the L9 cells show pacemaker activity, do not produce measurable EJPs in the gill (as recorded by an extracellular suction electrode), and send their axons to the gill via the siphon nerve. In addition, L9 cells are much less effective than L7 in producing gill movements.

Perfusion of the gill with the DA antagonist ergometrine maleate ($1\text{-}3 \times 10^{-4}$ M) produces at least partial and usually complete abolishment of L9-induced contractions. L7 and LDGl contractions are not affected. Fluphenazine HCl ($3\text{-}4 \times 10^{-5}$) appears to act as a mixed agonist-antagonist. That is, fluphenazine alone produces DA-like contractions, and it enhances L7-induced contractions. But fluphenazine blocks L9-induced contractions. LDGl contractions are not blocked by fluphenazine.

These results have led us to conclude that L7 is not dopaminergic and to develop the working hypothesis that the L9 cells are dopaminergic motor neurons. Biochemical assay of the L9 cells for DA will further test this hypothesis.

593 PHYSIOLOGY OF THE JUMP RESPONSE IN DROSOPHILA MELANOGASTER. Mark A. Tanouye* (SPON: J. Rosenbaum). Biol. Dept., Yale Univ., New Haven, Conn. 06520.

When a fly is startled, it initially jumps before opening its wings and flying away. The jump response is thought to be mediated via a giant fiber pathway from the brain to the thoracic ganglion[1]. The giant fiber system has been described anatomically from light and electron microscope serial sections[2]. The giant fiber synapses with a large motoneuron which innervates the tergotrochanter muscle of the ipsilateral mesothoracic leg, and an interneuron which innervates the motoneurons of the contralateral dorsal longitudinal flight muscles (wing depressors). In the present study, a physiological analysis of the jump response is described.

The mesothoracic leg provides the major thrust for the jump response. The tergotrochanter is a large, non-fibrillar muscle which originates on the dorsal surface of the thorax and inserts on the trochanter of the mesothoracic leg. The tergotrochanter may be selectively stimulated by an electrode located at its dorsal origin. In response to such stimulation, an all-or-nothing muscle potential is initiated, and the leg undergoes a fast and powerful extension. In addition, tergotrochanter activation causes an elevation of the wings.

The tergotrochanter muscle may also be activated by brain stimulation or cervical connective stimulation. In response to such stimulation, potentials may be recorded from the tergotrochanter muscle and the dorsal longitudinal muscles. These potentials have identical thresholds and characteristic latency relationships. In a dissected preparation, intracellular potentials were recorded from the giant fiber axon during brain stimulation. The results of these experiments show that tergotrochanter and dorsal longitudinal muscle activation due to brain stimulation occur via the giant fiber pathway.

Activity in the tergotrochanter muscle, the dorsal longitudinal muscles and the dorsal ventral muscles (wing elevators) was recorded at the start of normal tethered flight. Potentials occurred in the different muscles with latency relationships which were indistinguishable from those seen in response to brain or cervical connective stimulation. The present study therefore demonstrates that giant fiber activation alone is sufficient to explain the jump response of Drosophila melanogaster.

[1]Levine and Tracey (1973). J. comp. Physiol. 87:213-235.

[2]King (1976). Neurosc. Abstr. 2:628.

594 CALCIUM CURRENTS AND K-INACTIVATION MODULATE MOLLUSCAN SOMA SPIKES. S. H. Thompson* and P. A. Getting* (SPON: W. Craelius). Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305.

The duration of somatic action potentials in some identified molluscan neurons increases markedly in a use-dependent fashion during low frequency repetitive firing. Spikes in these cells are characterized by a distinct bump on the falling phase which broadens into a plateau during repetitive activity and results in up to a 2.3 times increase in spike width at a frequency of 1 Hz. Spike broadening and the bump on the falling phase of spikes are blocked in Ca-free-Co saline, suggesting that a Ca^{++}-dependent process is involved.

Under voltage clamp inward current was studied in 100 mM TEA saline and was separated into a transient Na-current and a delayed Ca-current by ion substitution methods. Those cells which show spike broadening have much larger delayed Ca-currents than other cells. Repetitive depolarization at 1 Hz results in a small, progressive decrease in both Na- and Ca-currents.

The net outward current decreases during repetitive depolarizations or maintained clamps. Outward currents decrease to a similar degree both in cells that do and in those that do not show spike broadening. The decrease in net outward current does not result from an increase in inward current, nor from extracellular potassium accumulation, but rather from cumulative inactivation of the voltage-dependent component of delayed outward current, termed K-current. K-current inactivation does not proceed according to the formalism used by Hodgkin and Huxley to describe Na-current inactivation in squid axon. Instead, K-current inactivation accumulates during repetitive pulses and does not recover significantly during the interpulse interval at a stimulus frequency of 1 Hz. The Ca-dependent component of delayed outward current, termed C-current, does not inactivate at low frequency.

Both delayed Ca-current and cumulative K-current inactivation are required for spike broadening. The summation of maintained inward Ca-current with a progressively decreasing outward current results in spike broadening during repetitive firing.

595 MORPHOLOGY OF IDENTIFIED NEUROMUSCULAR JUNCTIONS IN LOCUST JUMPING MUSCLE. M.J. Titmus* and G. Hoyle, Department of Biology,. University of Oregon, Eugene, OR 97403

The extensor tibiae of the jumping leg of the locust is innervated by four physiologically different neurons; fast excitor (FETi), slow excitor (SETi), common inhibitor (CI), and a neurosecretory neuron. Neuromuscular junctions (NMJ's) were identified and examined electron microscopically. FETi terminals were found by examining fibers known to be innervated only by FETi. CI and SETi endings were distinguished in fibers innervated only by them after stimulating one axon or the other in presence of horseradish peroxidase (HRP)-saline. After gluteraldehyde fix, 3-3'-diaminobenzidine-HCl-H_2O_2 incubation, and osmium tetroxide post-fix, terminals could be identified as CI or SETi by presence or absence of HRP-labeled vesicles.

Excitatory and CI terminals are morphologically different; the former having junctional contacts on granular material on the muscle fiber surface and the latter almost always having no granular material. Pre- and post-synaptic membrane thickenings, pre-synaptic dense bodies and bars occur at all three kinds of NMJ's. The synaptic gaps at excitatory junctions usually contain dense material whereas CI gaps contain little or none.

Morphologies of vesicles in the three types of NMJ's differ qualitatively and quantitatively. Maximum and minimum diameters and their ratios were determined by computer from data fed in directly from a graphics terminal. Vesicles in excitatory endings are nearly round (max. diameter (d.)/min. d. ratios: FETi = $1.16 \pm .14$, n=1408, 12 terminals; SETi = $1.15 \pm .14$, n = 1408, 14 terminals). FETi vesicles are significantly ($P<<.001$) larger (mean max = 59.9 ± 8.4 nm, mean min = 51.8 ± 7.3 nm, mean x-sec area = 2450 ± 592 nm^2) than SETi (mean max = 49.8 ± 6.0 nm, mean min = 42.8 ± 5.2 nm, mean x-sec area = 1684 ± 341 nm^2). CI vesicles are irregular (max. d./min. d. = $1.27 \pm .18$, n=1050, 11 terminals with HRP and $1.38 \pm .24$, n=896, 9 terminals without HRP) but have similar areas to those of FETi (2561 ± 501 nm^2).

These studies show that physiologically different NMJ's display different ultrastructures and also that fast and slow axon terminals, although they may use the same transmitter, contain vesicles of different size. Investigations are in progress to determine whether this size difference is reflected physiologically in size of single quanta. (This research was supported by NSF BMS75-00463 and PHS 5 T GM 00336)

596 A MODIFICATION OF THE PARAFORMALDEHYDE HISTOFLUORESCENCE TECHNIQUE FOR APLYSIA CENTRAL GANGLIA AND MUSCLE. Thomas K. Tomosky-Sykes. Div. Neurobiology & Behavior, Dept. of Physiol., College of P & S, Columbia Univ., New York, N.Y. 10032.

There has been difficulty in using the paraformaldehyde (PF) histofluorescence technique, which allows visualization of 5-HT and catecholamine neurons, in the central nervous system (CNS) of marine organisms. I have developed modifications of the technique which permit the localization of 5-HT and dopamine in Aplysia CNS and muscle and may be applicable to other marine animals. The pinned-out tissue is exposed to a 10% formalin/20% sucrose solution in artificial seawater for 5 minutes (Azmitia and Henriksen; J. Histochem. Cytochem., 24:1286-1288, 1976), then desiccator dried over phosphorous pentoxide for 4 days. Thereafter the tissue is sequentially exposed to 70% relative humidity PF for 1 hour and 90% relative humidity PF for another hour at 80° C. The tissue is examined as a whole mount in oil using incident UV illumination. Fluorescence specificity was checked using the sodium borohydride technique. Ganglia were sometimes pretreated with 6-hydroxydopamine to reduce dopamine fiber fluorescence.

In contrast to a previous method which was not optimal for visualizing cell bodies, 5-HT and dopamine cell bodies were found in all ganglia (except genital) which were examined. The serotonergic nature of the RB cells in the abdominal ganglion and of the metacerebral cells in the cerebral ganglion has been confirmed. Furthermore, 5-HT nerve fibers were found in the heart, and dopamine fibers were found in the penis and gill. This method is currently being used to test the hypothesis that facilitator cells of the gill-withdrawal reflex are serotonergic (Brunelli et al.; Science, 194:1178-1181, 1976).

597 IDENTIFIED NEURONS MAKE IDENTICAL SYNAPSES IN MUSCA AND DROSOPHILA. Karen L. Valentino* (SPON: R. J. Wyman). Yale University, New Haven, CT 06520.

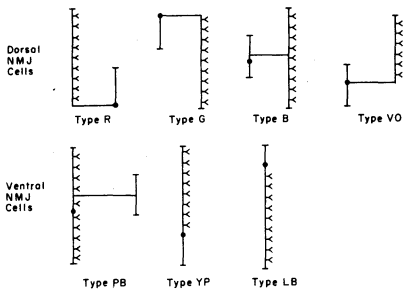
An interneuron has been found in Drosophila which synapses within the posterior dorsal mesothoracic nerve (PDM nerve) onto the dorsal longitudinal muscle (DLM) motor axons (King, Neuroscience Abstracts 3). It is thought that these synapses may mediate rapid conduction from the giant fibers to the flight muscles. If this is indeed the case, the physiological significance of the synapses in flight might be better studied in a larger fly. In fact, an interneuron similar to the one observed in Drosophila has also been found in Musca domestica.

The arrangement of the flight muscles and ganglion in the Musca thorax is virtually identical to that in Drosophila, although in Musca the anterior dorso-ventral fibrillar muscle is composed of four fibers rather than the three that are usually found in Drosophila. The major nerves are easily located in thick sections of the thorax. The proximal portion of the PDM nerve contains a synaptic region anatomically similar to that described in Drosophila. Although none of the nine large axons have been rigorously identified by serial section tracing to muscle motor end plates, each of the nine axons can be matched with axons appearing in corresponding sections in Drosophila. Three of these axons (the very large axon to the tergal depressor of the trochanter and the two accessory axons which also innervate this muscle) separate from the PDM nerve distal to the ganglion. The six other large axons represent the five DLM motor neurons which surround the peripheral interneuron. Synapses between the motor neurons and the peripheral interneuron appear to be formed by direct contact between the axons. The interneuron ends distal to these synapses, while the motor neuron axons increase in diameter as they continue toward the muscles.

The Muscidae are calypterate flies, while the Drosophilidae are acalypterates. The smallest taxonomic group that the two flies have in common is the suborder Cyclorrhapha. It is of interest, then, that such a specialized synaptic contact can be found in two such distantly related flies. This raises the possibility that this synapse may exist more widely within the order Diptera.

598 ELECTROPHYSIOLOGY AND ANATOMY OF IDENTIFIED NEURONS OF ASCARIS. J.P. Walrond,* I.S. Kass,* J.E. Donmoyer,* J.E.R. Moses* and A.O.W. Stretton Dept. of Zool., Univ. Wisconsin, Madison, WI 53706

The motor nervous system of Ascaris consists of five repeating sets of segmental neurons each containing eleven cells that make synapses onto muscle. In addition there are six ventral intersegmental neurons that make synapses onto motorneurons but not onto muscle. The motorneurons can be divided into seven classes on the basis of their shape and projections to muscle. 5 of the classes (R,G,B,VO and PB) send processes to the dorsal cord. The function of these five classes of motorneurons has been determined physiologically by stimulating single neurons and recording the evoked responses in the musculature. Of the five classes, 2 (B and PB) are inhibitory and three (R,G, and VO) are excitatory. The inhibitory neurons (one of which projects to the dorsal cord, the other to the ventral) receive their synaptic input from motorneurons, not from interneurons. This provides a pathway for reciprocal inhibition between the dorsal and ventral musculature. The excitatory neurons receive their synaptic input from the interneurons. (Supported by PHS Grant NS10509, NSF Grant BNS 76-09641, The Sloan Foundation, and The Research Fund of the Graduate School, Madison)



599 DURATIONS OF UNITARY SYNAPTIC POTENTIALS ARE MATCHED TO DIFFERENT DURATIONS OF INHIBITION REQUIRED BY A BEHAVIOR PATTERN. Jeffrey J. Wine and Grace Hagiwara*. Dept of Psych., Stanford U., Stanford, CA 94305.

Attempts to establish the neural bases of behavior invaribly rely on properties that emerge when neurons interact in large networks. In such accounts, the detailed cellular properties of neurons, such as membrane time constants, synaptic delays etc. are often obscured. Nevertheless, it is sometimes possible to relate basic cellular properties to behavior in a surprisingly direct way. We recently found that the durations of unitary synaptic potentials produced by different neurons are directly reflected in the temporal pattern of behavior.

Crayfish escape by rapid flexion and extension of the abdomen (a 'tailflip'). Flexion lasts for roughly 45 msec and is immediately followed by extension, which lasts another 60 msec, making the entire response just over 100 msec in duration. A tailflip can be triggered by a single impulse in one of four giant command neurons. The command neurons excite the flexor motoneurons directly, and thereby cause the flexion phase. In addition to their direct motor effect, the command neurons trigger widespread inhibition. Inhibitory postsynaptic potentials (IPSPs) have been recorded in eight classes of cells involved in tailflips. Five classes of cells in the flexion circuit (receptors, interneurons, command cell, giant flexor motoneuron, and flexor muscle cells) are inhibited within 10 msec after the initial flexion command, and remain inhibited during the entire cycle of flexion and extension. This ensures that flexion is brief and prevents its interference with reextension. Three classes of cells in the extension circuit (receptors, motoneurons and extensor muscle cells) are also inhibited following the flexion command, but they are then activated at the conclusion of flexion. Therefore, inhibition in the extension elements must be brief.

The requirement for long duration inhibition in flexor elements and short duration inhibition in extensor elements is met, in part, by synaptic specializations. Unitary IPSPs in flexor elements cause conductance increases lasting 70 msec or longer, while IPSPs in extensor elements are less than half that long. No exceptions were observed; long duration IPSPs (50 to > 100 msec) are seen in all five classes of flexor elements, short duration IPSPs (15-40 msec) are seen in all three classes of extensor elements.

A promising feature of this system is that four of the inhibitory neurons are identified. The transmitter in all cases is probably GABA, and all of the inhibitory synapses are accessible.

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600 MODULATION OF LOCOMOTOR ACTIVITY BY ADJACENT CAMPANIFORM SENSILLA VARIES WITH SENSILLUM ORIENTATION. Sasha N. Zill*, Francisco J. Varela* and David T. Moran*. (SPON. A.R.Martin). Dept. Anat., Univ. Colo. Med. Center, Denver, Co. 80262.

Campaniform sensilla are mechanoreceptors that respond to cuticular strain. In the cockroach, Periplaneta americana, leg campaniform sensilla reflexively modulate postural and locomotor activities. These sensilla are also directionally sensitive: they respond to compression perpendicular to the long axis of their ovoid cuticular caps. The tibial segment of the leg is unique in possessing two adjacent subgroups of sensilla of mutually perpendicular orientation. The sensilla of the proximal subgroup, oriented transverse to the axis of the leg, respond to passive tibial extension. Those of the distal subgroup, oriented parallel to the leg axis, respond to tibial flexion (Spinola and Chapman, J. Comp. Physiol. 96, 257-272, 1975).

To determine if their reflex effects are also orientation dependent, individual campaniform sensilla were stimulated with a fine tungsten wire probe. Reflex effects were monitored by extracellular recording of motoneuron activity in nerves to the extensor and flexor muscles of the trochanter; these muscles lower and raise the leg respectively. Single sensilla exhibited potent, selective reflex effects that strictly depended upon their orientation. Sensilla of the proximal subgroup increased activity of the slow excitatory axon to the extensor muscle (axon D_S) and decreased activity of excitatory axons in the flexor nerve. In contrast, the distal subgroup decreased activity of the extensor axon and increased activity of flexor axons. Simultaneous recording of another nerve containing a branch of the common inhibitory axon confirmed that its activity was not affected by stimulation of sensilla in either subgroup.

We are presently testing the hypothesis that these sensilla are selectively excited in different postural positions assumed by the animal and modulate centrally generated locomotor patterns accordingly. (Supported by NIH-BRSG RR-05337, NIH Training Grant GM-01981-07, and NSF Grant BMS-73-06766).

LIMBIC SYSTEM

601 A GOLGI STUDY OF CELL TYPES IN THE HILAR REGION OF THE RAT HIPPOCAMPUS. David G. Amaral. Dept. of Psychology and Center for Brain Research, Univ. Rochester, Rochester, N.Y.

In view of the long-standing debate about the identity of the cells in the hilar region of the dentate gyrus, namely whether they are to be regarded as a special subfield of the Ammon's horn (as Lorente de Nó believed) or as a component of the dentate itself (Cajal's *stratum polymorphe*) an analysis of the cells in this zone in the rat has been undertaken, using several variants of the rapid Golgi method. No fewer than twenty different neuronal cell types have been identified on the basis of their somal size and shape, the number and orientation of their dendrites, the presence and types of dendritic spines, and the distribution of their axons. The association of dentate granule cells with the cells in the hilar region was found to be more extensive than has previously been reported. Dentate granule cell axons appear to terminate on dendrites of non-pyramidal cells, which are devoid of "thorny excresences", by means of filiform extensions which originate from mossy fiber terminal expansions and by means of thin collaterals of mossy fibers. The cells throughout the hilar region show an impressive number and variety of complex dendritic appendages. It is concluded that this region is an area of mergence of polymorphic zones of the *cornu ammonis* and the *fascia dentata*. Moreover, the present analysis does not provide support for the contention of Lorente de Nó that the deep hilar region is an extension of the *stratum pyramidale*. Rather, it is in agreement with Blackstad's suggestion that the "CA_4 area" is in fact the polymorphic zone of an *area dentata*. In support of this position are the following (1) pyramidal-like cells above the CA_{3c} region lie nearly parallel to the *stratum pyramidale*, apparently these cells have become "reflected" off the CA_4 region; (2) the "modified pyramidal cells" of the CA_4 region are so qualitatively and quantitatively different in thorny excresence density and dendritic orientation and thickness as to clearly distinguish them from typical pyramidal cells.

602 CYTOGENESIS AND MORPHOGENESIS IN THE RAT SEPTAL REGION. Shirley A. Bayer. Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47906

Cytogenesis in selected nuclear groups of the rat septal region (diagonal band of Broca, medial, lateral, triangular, and septofimbrial) was analyzed autoradiographically after 3H-thymidine injections on two consecutive days during the prenatal period (5 μCi/gram body weight, day of sperm positivity is day 1). The percentage of cells labelled by each series of injections was determined in the offspring at 60 days of age in anatomically matched sections. By means of the progressively delayed comprehensive labelling method, the formation times of each cell population were determined.

Most of the neurons in the septal region form between days E15 and E17. There is a mediolateral gradient of cytogenesis within the major portion of the septum. The neurons in both medial septal and triangular septal nuclei form early (medial septal, 58% on E14-15; triangular septal, 56% on E15), and cytogenesis is essentially completed on E16. Neuron formation in both the lateral and septofimbrial nuclei occurs later, mainly on days E15-17 (lateral septal, 80%; septofimbrial, 74%). Formation is completed in the septofimbrial nucleus on E17, while it continues in the juxtaventricular zone of the lateral septal nucleus until E19. The neurons of the diagonal band also form on days E15-17 (69%); here, there is a caudorostral gradient of growth with anterior levels forming later than posterior levels.

The two gradients of growth seen in cytogenesis are also found in morphogenesis. The septal region develops from a ventromedial ridge of neuroepithelium along the lateral ventricle which continues across the midline to form the anterior boundary of the foramen of Monro. A zone of differentiating cells lies medial to the neuroepithelium on E14. In the region of the presumptive medial and lateral septal nuclei, this zone expands as new cells are added laterally. Concurrently, the neuroepithelium becomes progressively thinner, and mitotic activity drops off rapidly after E18. In the region of the presumptive diagonal band, there is a medial zone of differentiating cells from E15 on which lengthens caudorostrally. The germinal zone at this level contains both a neuroepithelium and a subependymal layer. Both remain active up to the time of birth and probably provide neurons for the olfactory tubercle, islands of Calleja, and nucleus accumbens.

603 PRESURGICAL HANDLING AND EXPLORATORY BEHAVIOR OF RATS WITH SEPTAL LESIONS. Wail A. Bengelloun[1], Jerry Finklestein*, Richard G. Burright* and Peter J. Donovick. Dept. Psychology, SUNY-Binghamton, Binghamton, New York 13901

The effects of septal lesions on behavior are greatly altered by the presurgical history of the experimental animal. Previous research from our laboratory indicates that environmental/social enrichment, dietary enrichment/deletion, and genotype-by-environment interactions all alter the classic septal syndrome. One form of early stimulation employed by many laboratories is handling. Such manipulations alter subsequent reactivity of the intact animal to novel stimuli in an open field and decrease adrenal corticosteroid levels. Using young adult rats, the present research examined the interactions between presurgical handling and subsequent septal lesions on postsurgical exploratory behavior in an open field.

One group of sixty-day old rats were handled twice daily for 20 days prior to surgery. The other group was left undisturbed during this period. Half of each presurgical treatment group received septal lesions, the others underwent surgical control procedures. On the 10th and 11th day after surgery all rats were tested in a dimly illuminated openfield for 5 minutes. The number of squares crossed and the number of times each animal stood up was recorded for each animal.

Control rats stood up more frequently than those with septal lesions and handled animals stood up more than their nonhandled counterparts. Numbers of squares crossed revealed a somewhat different picture. On both days control animals were more active than their brain damaged counterparts. However, on the test, presurgically handled rats with septal lesions were more active than nonhandled septals, whereas the handled controls were slightly less active than nonhandled controls. By the second day of testing, handled rats in both the lesion and control groups were more active than their nonhandled counterparts. These data further indicate that the apparent effects of septal lesions on behavior are differentially influenced by the specifics of the presurgical manipulations and the test situation.

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604 CORTICAL PROJECTIONS OF THE THALAMIC MEDIODORSAL NUCLEUS IN THE RABBIT. Robert M. Benjamin, Jan C. Jackson* and Gregory T. Golden*. Dept. of Neurophysiology, University of Wisconsin Medical School, Madison, Wisconsin 53706.

The cortical projection of the thalamic mediodorsal nuclear complex (MD) in the rabbit was mapped with retrograde horseradish-peroxidase and anterograde tritiated proline techniques. The projection field occupied the entire medial wall rostral to a mid-corpus callosal level, wrapped around the frontal pole onto the lateral convexity and tailed off caudally on the dorsal bank of the rhinal sulcus. The projection of the lateral approximately one half of MD, the half which does not receive olfactory input, was confined to medial cortex supplying all but its most rostral region. This projection field of lateral MD was precisely organized in two dimensions with the most lateral part projecting most caudally on cortex and the most dorsal part projecting most ventrally. A representation for the third, anterior-posterior (A-P), dimension was not evident since any cortical point within the field was supplied by a cylinder of cells extending the entire A-P extent of lateral MD. The medial half of MD, which does receive olfactory input, projected to the remaining rostral medial cortex, the lateral convexity and rhinal sulcal region. The inverse dorsoventral relationship was partially preserved and an overlapping A-P gradient was present with sulcal projections originating more caudally in medial MD and the rostral medial projection originating more rostrally.

The MD field on medial cortex was shared with projections from other thalamic nuclei. PT (parataenialis) projected to the medial MD field on the rostral medial wall. AM (anterior medialis) projections were essentially coextensive with the lateral MD field and, within this field, VA (ventralis anterior) supplied the region of the supplementary motor area.

605 AXO-AXONIC SYNAPSES ON INITIAL SEGMENTS OF HIPPOCAMPAL PYRAMIDAL CELLS. R.B. Chronister*, Dept. Anat., R.W. Sikes and L.E. White, Jr.*, Neuroscience Research, Col. of Med., Univ. of South Alabama, Mobile, AL 36688.

The existence of synapses onto initial segments of axons within the central nervous system has been documented in the prepiriform cortex. These synapses occur on spinelike processes of the axons of prepiriform cortex neurons. These spinelike processes are visible in both light and electron microscopy. Golgi impregnations of the hippocampal formation reveal similarly appearing spinous processes. For this reason, the hippocampal formation was examined with transmission electron microscopy to ascertain the presence of initial segment axo-axonic synapses.

Adult rats were perfused with Peters' modification of Karnovsky's fixative. Following fixation, small blocks were cut from precisely localized regions of the hippocampus and postfixed in 1% osmium tetroxide. The blocks were then stained en bloc in uranyl acetate and embedded in epon. One micron thick sections were cut and examined to further substantiate localization. Silvergray sections were mounted on grids, stained per usual with lead citrate, and examined on a Phillips 301 transmission electron microscope. Initial segments were recognized by the thickening of the membrane and the bundling of the microtubules.

The spinous processes were of two basic types. The most common spine was sessile although pedunculated spines were also encountered. The pedunculated spines had vacuolated structures similar to those of dendritic spines while the sessile spines appeared to be devoid of these structures. In addition to the vacuolated structure, the pedunculated spines also contained fine filaments.

Both types of spines were postsynaptic. Presynaptic boutons often contained flattened vesicles and made type II synapses upon the spines. Similar type II synapses also were found on the shafts of the axons. On the dendrites of these neurons, type I synapses were present on the dendritic spines. The significance of these findings will be discussed.

606 Effects of transecting septal and subicular afferents to the anterior thalamic nuclei on straight-alley performance. Robert E. Davis, Janice Hendricks, and Ernest W. Kent. Dept. of Psych., University of Ill. at Chicago Circle, Chicago, Ill. 60680

Previous research suggests that the anterior thalamic nuclei receive direct input from the septum (primarily the lateral septum nucleus) and the dorsal subiculum (Meibach and Siegel, 1976). These fibers are susceptible to destruction by knife-cuts in the coronal plane, posterior to the columns of the fornix, ventral to the stria medullares, dorsal to the ventral fornix, and extending 1.5 mm on either side of the midline (Davis and Kent, 1976). This damage results in behavioral changes similar to those seen following total destruction of the hippocampus, septum or dorsal fornix (i.e., hyperactivity and enhanced acquisition of bidirectional shuttlebox avoidance). In an extension of this work, we have investigated the effects of this transection on the performance of rats in the straight alley.

Preoperatively, animals were trained to stable baselines. Following this, animals received either knife-cuts (AP-6.6, H-0.0, L-1.5; de Groot) or sham operations. After running speeds stabilized, the response to change in the amount of reward (shifting from 3-8-1-3 pellets; 3 days at each level), in the tactile cues of the alley and in the olfactory cues of the goal box were examined in these animals. Sham operated control and transected groups were similarly disrupted by both tactile and olfactory cue manipulations. Running speeds for both groups were initially elevated, returning to normal levels after several trials. Only sham operated animals were affected by changes in the amount of reward (downshifts from 8 to 1 pellet produced maximal disruption). Knife-cut animals were totally unresponsive to either downshifts or upshifts in the amount of reward. Running speeds for this group did not deviate from previously established baselines across all levels of reward.

Additionally knife-cut animals required significantly more trials than controls to reach an extinction criterion following removal of appetitive reward from the goal box.

607 DIFFERENTIAL EFFECTS OF AMYGDALA AND HIPPOCAMPUS STIMULATION ON OPERANT BEHAVIOR. Russell Denea*, Henry Lesse and Jeremiah Collins*. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

The effects of disruption of amygdala and hippocampal function by focal stimulations were studied in cats trained to bar press (BP) for milk. Since both structures have very low thresholds for epileptiform afterdischarges (AD), the relationship between electrographic and behavioral responses to repeated stimulations was investigated. During low frequency stimulation, continuous bilateral recordings from amygdala, hippocampus, neocortex and other sites were used to monitor brain activity; BP rate was recorded simultaneously. The threshold for eliciting afterdischarges (ADT) in the structure stimulated was determined by gradually increasing current while other parameters were held constant. Then ADT tests were continued at 48-hr intervals until threshold values stabilized.

Stimulation of the amygdala at intensities subthreshold for local afterdischarges and for elicited motor responses resulted in marked suppression of operant behavior which progressed as current was increased. In contrast, hippocampal stimulation proved significantly ($p < .001$) less disruptive of task performance at all intensities. The contrast was even more striking when ADs were induced. During 50 amygdala stimulation sessions (7 cats) there were no instances of bar pressing when ADs were evoked. However, in about half of the hippocampal ADT tests, subjects continued to BP during ADs. Although there was a mean decrease in BP rate during these sessions, the performance of some subjects was unchanged during induced ADs. In both structures, repeated focal stimulations resulted in an initial rapid decline in ADT with stabilization by the 7th session. A concomitant reduction in the currents required to disrupt behavior occurred indicating a parallel increased sensitivity to the effects of subthreshold stimulation on learned behavior. After subsequent repeated stimulations generalized motor seizures, rather than local ADs, were elicited eventually. The differential behavioral effects of subthreshold stimulation of the amygdala and hippocampus persisted after these "kindled" seizures developed.

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608 A HORSERADISH PEROXIDASE STUDY OF SUBCORTICAL PROJECTIONS TO THE HIPPOCAMPAL FORMATION IN SQUIRREL MONKEY (SAIMIRI SCIUREUS). J. L. DeVito. Reg. Primate Res. Ctr., Univ. of Wash., Seattle, WA 98195

Large amounts of horseradish peroxidase (0.7-1.4 µl; 33%) were injected into rostral and caudal regions of the hippocampal formation involving the hippocampus, entorhinal cortex and amygdala rostrally and hippocampus, entorhinal cortex and retrosplenial cortex caudally. More discrete amounts (0.2 µl; 33% HRP) were injected into the middle part of the hippocampus, subiculum and presubiculum. A control needle track descending as far as the lateral geniculate nucleus was injected with 0.2 µl HRP.

Sites of retrograde transport common to all hippocampal injections were as follows: the perifornical region in the dorsal hypothalamus, the supramamillary nucleus, the lateral hypothalamic area at the level of the mamillary bodies, the medial septal area including dorsal and ventral components of the diagonal band nucleus and the anterdorsal (AD) and laterodorsal (LD) thalamic nuclei. The large caudal hippocampal injection resulted in a large number of labeled cells in LD and AD and a smaller number in the anteroventral (AV) thalamic nucleus. Large rostral injections resulted in labeled cells in the anteromedial (AM) and anteroventral thalamic nuclei and fewer labeled cells in AD and LD. With the exception of one case limited largely to an injection of the dentate gyrus, subiculum and presubiculum, labeled cells were present in the midline tegmentum inferior to the decussation of the brachium conjunctivum. The dorsal raphe nucleus was labeled in all cases but also exhibited a few labeled cells in the control animal. Midline thalamic nuclei were labeled after large rostral and caudal injections but not after more restricted injections.

These results reveal tegmental and hypothalamic sources of afferent fibers to the hippocampal formation. The generally accepted medial septal and diagonal band nuclei projections were readily apparent. However, a projection from the lateral septal area which has been suggested recently (Siegel and Tassoni, Brain Behav. Evol., 4:185, 1971) was not observed. Finally, the anterior thalamic nuclei and the laterodorsal thalamic nucleus appear to project to the hippocampal formation, possibly via the cingulum bundle (Domesick, Brain Res., 20:19, 1970).

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609 HABITUATION OF AROUSAL IN RATS WITH SELECTIVELY REDUCED HIPPOCAMPAL CONNECTIONS. Mary W. Dickie* and Phillip J. Best. Dept. of Psychology, Univ. Virginia, Charlottesville, VA 22901

The hippocampus is important in a wide variety of behaviors including habituation of the arousal reaction. Hippocampal unit activity is drastically reduced when the animal is awakened from slow wave sleep (SWS) (Mays and Best, Exptl. Neurol. 1975, 47, 268-279). This arousal habituates over time. Lesions of the fimbria-fornix (FF) or of the entorhinal cortex (EC) enhance duration of arousal from SWS and delay habituation (Wacker and Best, NSC 1976 #530). The current study asks how habituation of the arousal response is affected by simultaneous bilateral lesions of both EC and FF.

On a related issue, Steward, Cotman and Lynch (Exptl. Brain Res. 1974, 20, 45-66) report that unilateral lesions of the entorhinal cortex cause new fibers to sprout from the contralateral dentate gyrus within 8-12 days. The current study asks what effect a unilateral EC lesion will have on habituation of the arousal response. Furthermore, will the nature and magnitude of such an effect change over time with the appearance of newly sprouted fiber projections.

Fifty four male Sprague-Dawley rats were grouped as controls (C) and as rats sustaining electrolytic lesions as follows: bilateral entorhinal cortex (EC), fimbria-fornix (FF), bilateral entorhinal cortex and fimbria-fornix (BF), and unilateral entorhinal cortex subdivided into immediately-tested (UI) and delay-tested (UD) groups.

EEG activity was recorded from neocortex and hippocampus. All groups were tested following 3-day recovery except UD group which was delayed 21 days to allow for sprouting. A series of 25 tones (2.9 KHz, 89 dB, 1 sec) was presented during SWS. The arousal response was assessed on behavior and on neocortical EEG activation following each tone.

Both FF and EC groups showed an initial arousal response higher than C group, both showed significant resistance to habituation ($p<.05$), and EC's showed persistently higher levels of arousal than FF or C. The BF group showed the highest level of arousal throughout. The UI group had a higher initial arousal response than C or UD but did not differ from them during later tones. Groups C and UD did not differ.

Thus, partial interruption of hippocampal connections by either FF or EC lesions enhance arousability, and attenuate habituation of the arousal response. Compound lesions result in even greater arousal which is strongly resistant to habituation. Unilateral EC lesions do not produce a measurable deficit. Thus, minor damage of hippocampal connections does not cause appreciable loss of habituation of the arousal response.

610 SELECTIVE ATTENTION FOLLOWING SEPTAL LESIONS. Peter J. Donovick, Richard G. Burright*, William W. MacLaughlin* and Melinda K. Hull*. Dept. Psychology, SUNY, Binghamton, N.Y. 13901.

The discrimination behavior of rats with septal lesions is affected by their altered reactivity to stimulus conditions surrounding the learning situation. Compared to control animals, rats with septal lesions are differentially affected by experience with cues relevant to the task itself. Using original discrimination problems with relevant redundant cues, and then selectively making certain cues irrelevant, we recently showed that rats with septal lesions, relative to intact controls, use one cue-dimension at the expense of others. Such results imply an attentional rigidity on the part of septal damaged rats -- an interference with the mechanism's underlying the organisms' abilities to "take a multiple look" at its environment.

In the present experiment we examine the effects of septal lesions on how the rat responds to, and subsequently utilizes, cue information which was made relevant after they had achieved criterion performance in a discrimination task. Our results suggest that septal lesions result in a disruption of attention. That is, relative to control animals, rats with septal lesions apparently failed to attend to the newly relevant cues provided and clearly, were deficient when later called upon to utilize such information. These findings are consistant with our notion that septal lesions alter the relative weighting of important sensory information which the CNS must process, and that changes in learning performance frequently reflect a function of altered attentional mechanisms.

611 RELEASE OF ACETYLCHOLINE FROM CEREBRAL CORTEX AND HIPPOCAMPUS OF FREELY MOVING RATS. J.D. Dudar*, J.C. Szerb, Dept. of Physiology and Biophysics, Dalhousie University, Halifax, N.S., I.Q. Wishaw*, Dept. of Psychology, University of Lethbridge, Lethbridge, Alta.

Electrical recordings from the hippocampus show that rhmthmical slow activity (RSA or theta) is generated during voluntary movement (freq. 6-12 Hz) and in response to sensory stimulation (freq. 4-7 Hz). The former is resistant to atropine but blocked by urethane anesthesia, while the latter is preserved during urethane anesthesia and is blocked by atropine. In the neocortex low voltage, fast activity (LVFA) accompanies voluntary movement and this LVFA is sensitive to urethane but resistant to atropine, while the LVFA during sensory stimulation is preserved during urethane anesthesia but blocked by atropine. There appear to be two ascending systems: one activated by sensory stimulation and atropine sensitive, the other closely related to voluntary movement and atropine resistant (Vanderwolf, J. Comp. Physiol. Psychol. 88, 300, 1975).

In order to test whether these differences in EEG patterns reflect differences in the activation of cholinergic neurons, acetylcholine (ACh) was collected from the hippocampus (HC) and neocortex (CTX) of freely moving male rats during motor behavior (treadmill running), sensory stimulation and resting conditions. ACh was collected by a constant perfusion technique (0.02 ml/min) after acetylcholinesterase inhibition and using local atropine SO_4 (1 µg/ml) to maximize ACh release which was estimated by radioenzymatic assay.

Sensory stimulation and motor behavior induced similar increases in ACh release (2x resting levels) from the HC and the release was not changed by atropine sulfate injection (7 mg/kg i.p.). ACh release from CTX did not show significant changes. In the HC, sensory stimulation, under urethane anesthesia, induced a similar increase to that seen without anesthesia whereas drinking behavior did not alter ACh release above resting levels.

These results suggest that activation of cholinergic neurons in the HC does not correlate with the susceptibility of RSA to antimuscarinic agents. (Supported by M.R.C.).

612 THE ISLANDS OF CALLEJA. James H. Fallon, Joseph N. Riley, Jack C. Sipe and Robert Y. Moore. Dept. Neur., UCSD, La Jolla, CA 92093.

The islands of Calleja are a prominent and consistent feature of the mammalian brain, yet very little is known about their anatomy, connections or functions. The anatomy and connections of the islands were analyzed in the rat using Nissl, Golgi-Kopsch, and EM methods, the glyoxylic acid (GA) histochemical method, autoradiography and the horseradish peroxidase (HRP) technique for anterograde and retrograde tracing.

The islands of Calleja, as defined here, are composed of tight clusters of granule cells with an approximate diameter of 7µm and a nucleus which fills about 75% of the cell. The islands include the insula magna, located at the border between the septum and nucleus accumbens, and granule cell clusters in the polymorph layer of the olfactory tubercle (OT). The islands are surrounded by rich fiber plexi and frequently surround a central "core" of neuropil in which there may be a large neuron. In Golgi material, the granule cell axons are fine with small varicosities. The dendrites and axons ramify predominantly within the island cluster and adjacent neuropil and have not been seen to project out of the surrounding neuropil. The dendrites of medium and large cells in the surrounding polymorph layer of the OT, as well as cells of the septum and nucleus accumbens, penetrate into the islands, those of some nearby, larger cells appear preferentially to project into the islands.

Some afferents to the islands have been identified. These are topographically organized in a medial-lateral fashion. The afferents are from the dopamine (DA)-containing cells of the substantia nigra-ventral tegmental area (SN-VTA), the septum, nucleus accumbens, amygdala and piriform cortex. The dopamine innervation of the islands, as determined with GA technique, is moderately dense. The DA fibers can be found mainly in the neuropil surrounding the islands and in the "core" of the islands. Following injections of ^{3}H-proline/leucine into the SN or VTA, autoradiography demonstrated that the medial SN projects to the lateral islands, while the VTA projects to the medial islands. Injections of HRP into the septum, nucleus accumbens, dorsal amygdala or piriform cortex result in anterograde and retrograde labeling in the islands. Preliminary ultrastructural analysis of HRP material suggests, in accord with the light microscopic observations, a reciprocal projection between the granule cells and other basal forebrain structures. It is concluded that the granule cells and surrounding large cells may form additional loops in the basal forebrain, with the appropriate connections present to allow for significant integrative functions. Supported by USPHS NS-05187, USPHS NS-12080 and the Veterans Administration.

613 HIPPOCAMPAL COMPLEX-SPIKE AND THETA CELL ACTIVITY EVOKED BY STIMULATION OF LIMBIC STRUCTURES IN UNRESTRAINED RATS. S.E. Fox and J.B. Ranck, Jr. Neuroscience Prog., Univ. of Mich., Ann Arbor, MI. 48109 and Dept. of Physiology, Downstate Med. Ctr., SUNY, Brooklyn, N.Y. 11203

Three independently moveable recording microelectrodes were implanted stereotaxically in hippocampal formation of rats to hit the cell layers of CA1, CA3 and fascia dentata in a single lamella. Stimulating electrodes (fixed or moveable) were implanted in medial septal nucleus, in ventral hippocampal commissure (1 mm from the midline contralaterally) and in ipsilateral entorhinal cortex. These were positioned under electrophysiological control for maximal field potentials (FPs) in hippocampus at low thresholds. In addition, neocortical electrodes, neck muscle EMG electrodes and 1 to 3 fixed hippocampal reference electrodes were implanted and cemented in with acrylic. After a week, responses were recorded to single and paired stimuli to the various sites, taking care to keep behavior constant (i.e. in a relatively steady state). With this preparation most of classical hippocampal extracellular electrophysiology can be done in a behaving animal, allowing study of: 1) effects of different behaviors on electrophysiological characteristics of a given cell or FP, or 2) differences in electrophysiological characteristics of cells of different behaviorally identified types.

In most cases FPs in the freely moving animals were similar to those described by others in acute preparations. The behaviors used most extensively were slow wave sleep, paradoxical sleep, drinking and walking on a treadmill. Quantitative differences in both evoked unit activity and FPs were seen in several sites during the different behaviors, demonstrating the necessity for "steady state" behavior. We have previously reported that complex-spike (CS) cells and theta (T) cells differ in pattern and frequency of firing, duration, behavioral correlates and localization. Our present data showed: 1) at all locations, the latency of evoked CS cell activity was almost entirely predicted by the latency of the population spikes (presumably summed extracellular action potentials of projection cells--i.e. pyramidal and granule cells), while T cells had multiple action potentials before, during and/or (most frequently) after the population spikes; 2) CS cells were occasionally antidromically activated, T cells were not; 3) many T cells had additional electrophysiological properties of the "inhibitory interneurons" of Andersen, et al. (J. Neurophysiol. 27: 608, 1964). The present data, along with earlier data from this laboratory support the hypothesis that most CS cells are projection cells and that T cells are interneurons mediating recurrent inhibition.

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614 HIPPOCAMPAL RHYTHMICAL SLOW ACTIVITY (RSA) DURING OVERTRAINED LOCOMOTION AND JUMPING IN THE CAT. Christopher J. Frederickson, Kay L. Hall*, Charlotte S. Smylie* and John P. Drobnica*. Program in Psych., Univ. of Texas at Dallas, Box 688, Richardson, Tx. 75080.

From 9 cats chronically implanted with either fixed or roving macroelectrodes aimed at dorsal hippocampus, 4 cats which showed high-amplitude (350 to 600 μv; monopolar derivation) RSA in the hippocampal EEG were selected as subjects. The animals were trained to wait immobile till a door opened, then walk 3.2 M through an enclosed square alley to food presented on a continuous reinforcement (CRF) schedule. Hippocampal RSA accompanied locomotion in the alley (walking and trotting) through all phases of the study, during initial training, and after prolonged overtraining as well. On the last recorded session, after 4 to 21 wks of overtraining with 1037 to 3810 practice trials, RSA was present during locomotion in 86%, 98%, 100% and 100% of the seconds of record scored for the 4 animals; median amplitudes of the 100 RSA waves measured were 320, 440, 520 and 520 μv for the same 4 animals, respectively, and modal RSA frequencies were 6, 6, 7 and 6 Hz. Comparison of the data from the last recorded session with that obtained after only 1 wk of training showed no tendency for either frequency or amplitude of RSA to decrease with continued practice at the task. However, in 2 cats, RSA amplitude was slightly larger on the first training day than after 1 wk of training.

RSA of a slightly (1 Hz) lower modal frequency than that recorded during locomotion was also usually present during immobile waiting prior to walking trials; typically, the RSA during waiting was 5 to 15% larger in amplitude than that recorded during locomotion.

In a second experiment the same 4 cats were trained to jump .9 M to a platform for food (CRF). Examination of videotapes, and force-sensor records showed that the jumps were quite stereotyped after 5 to 10 trials. Hippocampal RSA was prominant during the last 1 to 3 sec prior to lift-off during initial training on the jump and remained prominant on virtually every trial even after lengthy (up to 2 wks with 500 jumps) overtraining.

The results suggest that hippocampal RSA activity accompanies the behaviors of walking, trotting, and jumping in the cat even after the behaviors have been thoroughly practiced and have become stereotyped in performance.

615 DIFFERENTIAL NOREPINEPHRINE AND SEROTONIN CONCENTRATIONS IN THE HIPPOCAMPUS OF NORMAL RATS AND IN RATS FOLLOWING SEPTAL LESIONS. Fred H. Gage, Roy G. Thompson* and James J. Valdes.* Chemistry of Behavior Program, TCU, Fort Worth, Texas 76129.

Fluorometric analysis of serotonin (5-HT) and norepinephrine (NE) content of the hippocampal formation revealed that both biogenic amines are distributed heterogeneously in the dorsoventral axis, and that NE also exhibits a heterogeneous distribution in the medial-lateral direction while 5-HT does not. Dissection of the hippocampus into its dorsal and ventral halves shows that both NE and 5-HT exhibit higher concentrations in the ventral hippocampus in comparison to its dorsal counterpart. A dissection which separated the cell fields CA 1&2 from CA 3&4 and the dentate gyrus showed NE to be highest in the latter region, while 5-HT was uniformly distributed between the two regions. Taken together, these data indicate that NE is more highly concentrated in the CA 3&4 and dentate area of the ventral hippocampus while 5-HT concentration differences are apparent only in a dorsal-ventral dissection.

Concentrations of NE and 5-HT in the dorsal and ventral hippocampus were also determined at 1, 3, 6, 11, 16, 24, and 30 days following a lesion to the septal nuclei. The results demonstrate that biogenic amine levels in the dorsal hippocampus achieve maximal depletion earlier than do their ventral counterparts, and that percent depletion is greater for 5-HT than NE in both dorsal and ventral areas.

On the first day following septal lesions, dorsal 5-HT is increased above normal levels. Sixteen days after a septal lesion, 5-HT is substantially depleted below normal levels. In addition, by 30 days, 5-HT shows recovery from its earlier depleted state. Behavioral changes related to sensory reactivity correlate with the relative decreases and recoveries of NE and 5-HT following septal lesions.

616 COMPARATIVE STUDY OF FOREBRAIN AFFERENTS TO THE HABENULA IN THE CAT AND THE RAT AS REVEALED BY HRP NEURONOGRAPHY. S. Gravel*, R. Boucher*, J.M. Scarabin* and A. Parent, Lab. Neurobiol., Fac. Med., Laval Univ., Quebec, Canada

Injections of HRP (30% sol'n, 0,05-0,4 μl) were made in the habenular complex of 11 cats and 13 rats. Retrogradelly labelled cells were visualized after a survival period of 24 to 48 hrs by means of the method of LaVail et al., ('72, '73). In rats, most injections were confined to the lateral habenular nucleus (LH). In such cases a very large number of labelled cells were found within the rostral two-thirds of the entopeduncular nucleus (EN) (see also Herkenham, Neurosc. Abstr., '75). Numerous positive neurons also occurred in the lateral preoptico-hypothalamic region (LPHR) and in the perifornical area rostrally to the level of the injection site. In addition a few labelled cells were found in the substantia innominata (SI), in the vertical limb of the diagonal band of Broca (DBB), beneath the ventromedial hypothalamic nucleus and at the basis of the claustrum. In cats most of the HRP injections were also confined to the LH. In such cases, numerous labelled cells were found in the posterior septal nucleus, in the bed nucleus of the stria terminalis and in the LPHR rostral to the injection site. In addition a few labelled cells occurred in the LPHR contralateral to the injection site. A significant number of positive cells were also present in the SI and/or the horizontal limb of the DBB and in the perifornical area. A few positive neurons were visualized in the vertical limb of the DBB, in the medial hypothalamus and in the ventral thalamus immediately above the third ventricle. The EN of the cat, however, contains very few labelled neurons although some positive cells of the LPHR closely surround the ventromedial edge of the EN. In a few cases where the injection site slightly invades the mediodorsal thalamic nucleus, numerous labelled neurons occurred in the olfactory tubercle and less abundantly in the accumbens septi nucleus. These findings suggest significant species differences regarding the habenular afferents in cats and rats. One of the most striking differences is that the EN of the cat contains very few HRP labelled cells after LH injection in comparison to the EN of the rat which can be shown to be the major source of afferents to the HL with the same method. The pallido-habenular projection in the cat, therefore, could be a much more discrete and/or a more highly collateralized system than it is in the rat.

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617 LOCALIZATION OF HIPPOCAMPAL SLOW ACTIVITY IN THE URETHANE-ANESTHETIZED RAT. Kenneth F. Green, J.N.P. Rawlins, and Per Andersen. Dept. Psych., Cal. State Univ., Long Beach, CA 90840, Dept. Exp. Psych., Oxford Univ., Oxford OX1 3UD, England, and Inst. Neurophysiology, Univ. of Oslo, Karl Johans Gt. 47, Oslo, Norway.

The purposes of the work were to map areas within the hippocampus where theta activity was generated and to examine phase relations between systematically selected sites.

Urethane-anesthetized rats were prepared by removing the skull and tissue overlying the left dorsal hippocampus and bathing the exposed brain in warm mineral oil. Theta was activated by stimulating the brain stem with tungsten microelectrodes. Recordings were made with silver ball electrodes on the surface or with tungsten microelectrodes during vertical or horizontal penetrations: one electrode remained stationary for gauging the momentary state of the animal; the other moved from the fimbria posteriorly or from the septum laterally in 0.5-mm steps.

Theta was found only in CA1, with amplitude maxima in stratum oriens (usually within the first 0.1 mm of penetration) and in stratum lacunosum-moleculare (at the fissure) separated by a null-theta region high in stratum radiatum. On occasion, each generator appeared capable of operating without the other. Below the fissure, a high-frequency activity masked theta, while in CA3 no theta was recorded unless cerebrospinal fluid was in contact with the conducting portion of the electrode.

Phase changes were found with vertical, lateral, and posterior movements of the electrodes. Vertical penetration revealed a shift of approximately 180° from surface to deep generators -- from + to - 0.1 mm of the null, a shift of about 140° was found in the direction of deep leading. Lateral movement showed no shift in the surface generator, but in the deep one medial sites led lateral sites by 20° to 30°. Movement anteriorly from the subiculum revealed a complex pattern in both generators: generally, more anterior sites followed more posterior sites; however, within 1.0 mm of center, an inversion occurred which persisted for about 1.0 mm in each generator. The inversion was more anterior in the deep generator.

618 COMPARISON OF SPONTANEOUS AND STIMULATED HIPPOCAMPAL THETA ACTIVITY IN RATS. Nobuyoshi Hagino and Hideo Saito. Dept. of Anatomy. U.T.H.S.C.S.A., San Antonio, Texas 78284.

Concentric bipolar electrodes were inserted stereotaxically into the frontal cortex, dorsal hippocampus (D-HPC), midbrain reticular formation (mdRF) and ventral region of periaquaductal gray matter behind the trochlear nucleus (PGM). Surgical preparation was performed under urethane anesthesia(900mg/kg body weight). Four hours after initial anesthesia sleep and arousal patterns were assessed in adult male rats by EEG recording from the frontal cortex and D-HPC. Stimulation of mdRF or PGM produced cortical arousal and hippocampal theta activity: arousal threshold was 0.25 volt 0.5 msec 100Hz for 10 sec. In these rats posterior thalamic deafferentation was performed using an L shaped knife; the middle and posterior thalamic nuclei were isolated posteriorly from the midbrain, and laterally from the internal capsule, and ventrally from the dorsal margin of the hypothalamus. In this preparation, the anterior region of thalamus and reticular hypothalamic pathways(Anchel,Lindsley 1972) were intact. Posterior thalamic deafferentation prevented spontaneous cortical arousal and hippocampal theta activity. Stimulation of mdRF or PGM was capable of inducing cortical arousal and hippocampal theta activity, however, the arousal threshold was elevated (1.0 volt).

An attempt was then made to determine functional pathways for inducing hippocampal theta activity. In intact rats stimulation of mdRF or PGM produced fast and slow components of evoked potential in D-HPC (fast component was determined within 10 msec after stimulation). After posterior thalamic deafferentation the fast component was suppressed and the pattern of slow component was altered upon mdRF stimulation. However, deafferentation did not alter the pattern of fast component upon PGM stimulation. Further extracellular unit recordings were made under urethane anesthesia in order to examine the fast component of evoked potential previously observed in D-HPC. Stimulation of PGM produced evoked unit responses which were superimposed on the fast component of evoked potential. However, mdRF stimulation did not produce such evoked unit response within 10 msec. The evidence indicates that PGM has a direct neural connection with D-HPC. However, mdRF may not have such a direct neural connection with the D-HPC.

These results and facts support the interpretation that diffused reticular thalamic pathways (Moruzzi, Magoun 1949) are responsible for spontaneous hippocampal theta activity. However, stimulation of the reticular hypothalamic pathway or periaquaductal septohippocampal pathway is capable of inducing hippocampal theta activity. Neither of these pathways are capable of inducing spontaneous hippocampal theta activity without involvement of the thalamus. (Supported by NIH HD 10071).

619 REINFORCEMENT VALUE AND SOCIAL BEHAVIORAL INFLUENCE OF ELECTRICAL STIMULATION OF THE HYPOTHALAMUS OF RHESUS MONKEYS. James G. Herndon, Jr.*, Adrian A. Perachio and Michael McCoy*. Yerkes Primate Center, Emory Univ., Atl. GA 30322 (SPON: W. Bouris).

Social aggressive behavior of rhesus monkeys can be elicited by electrical stimulation of the hypothalamus (Perachio and Alexander, In: Neuropsychology of Aggression, 1974). It has been argued that brain stimulation produces aggression only if the stimulation is aversive (Plotnik et al., In: The Physiology of Aggression and Defeat, 1971). While operant responding for stimulation of an attack-producing site was reported (Robinson et al., Physiol. Behav., 4, 1969), it has been pointed out that the duration of the stimulation trains which produced rewarding effects was considerably shorter than the duration used to produce attack (Valenstein, Nebr. Symp. on Mot., 1974). The present study assesses the social effects of short trains of stimulation of demonstrated reinforcement value.

Two male monkeys in whom social aggression was produced by 4-10 sec trains of stimulation applied to hypothalamic sites, were trained to press a lever for stimulation of other sites. Except for duration, the parameters of stimulation used as an operant reinforcer were identical to those used in the social setting (1.0 msec pulses at 50/sec, .3-.4 mA). Mean response rates for stimulation were: for animal #1, 15.8 resp/min, and for animal #2, 72.9 resp/min. When an attack-producing site was stimulated in the operant setting, animal #1 consistently maintained low-to-moderate rates of responding (5.62, range 3 to 20 resp/min). Animal #2, however, would not respond for stimulation of attack-producing sites. The following conclusions are drawn: (1) Moderate levels of operant responding can be maintained by stimulation of hypothalamic sites which produce attack. (2) Maintenance of responding for stimulation of attack-producing sites does not occur in all animals. Therefore, the reinforcement properties and social consequences of stimulation can be dissociated.

To determine the social consequences of short duration electrical stimulations, widely spaced .4 sec trains of stimulation were presented to animal #1 in a social setting. The animal alerted, oriented toward and initiated movement in the direction of the target animal. Thus, components of attack were apparent. Second, the rate of self-stimulation was tape-recorded to be reproduced in the social setting. While this experiment has not been completed, it seems likely that attack will occur since some clusters of recorded operant responses were dense enough to produce virtually uninterrupted stimulation, lasting several seconds longer than this animal's usual attack latency.

620 FORNIX FIBERS, CONTEXTUAL RETRIEVAL, AND MOTIVATIONAL STATES AS CONTROLLERS OF BEHAVIOR. Richard Hirsh, Brian Leber*, and Kelvin Gilman*. Dept. of Psychology, McGill University, Montreal, P.Q., Canada.

It is known that motivational states guide, as opposed to drive behavior in a manner that is different from external stimuli. It has been postulated that motivational states serve as retrieval cues and that this process is mediated by the hippocampus. As a test animals with total fornix transections or medial septal lesions were trained to obtain food and water in the same T-maze by making opposing responses. At the outset of learning both lesioned groups showed greater response consistency than sham-operated controls in terms of frequency of occurrence and alternation. This tendency increased and then decreased as a function of experience. The animals with transected fornices showed strong negative correlations between performance levels in obtaining food and water and slower in reaching criterion. Animals with septal lesions reached criterion more quickly than controls.

In another experiment the same type of animals were run in an experiment which was similar except that food and water were obtained in different mazes. The lesioned animals again showed response consistency during the early stages of learning, but reached criterion in a normal number of trials. The first experiment was repeated with animals in which the dentate granule cells had been destroyed by neonatal irradiation, with results similar to those of fornix transection.

The results support the proposed involvement of the hippocampus in the process by which motivational states prompt retrieval.

621 SUBCORTICAL CONNECTIONS FROM AREA 9 WITHIN THE PRINCIPAL SULCUS OF THE RHESUS MONKEY CEREBRUM. Stanley Jacobson, Nelson Butters* and Nisa Tovsky*. Department of Anatomy, Tufts University School of Medicine and Psychology Service, V.A. Hospital, Boston, Mass., 02111.

In the rhesus monkey removal of the midportion of the cortex around the principal sulcus produces impaired ability to perform delayed alternation tasks. In order to more completely define the anatomical connections of this region, the retrograde and anterograde axoplasmically transported tracers (Horseradish peroxidase and tritiated proline, lysine and leucine) were injected into the cortex surrounding the principal sulcus. In three adult rhesus monkeys the tracers were injected into the dorsal and ventral portions of the cortex bordering the principal sulcus with animal one receiving injections into the anterior one-third, animal two receiving injections into the middle third and animal three receiving injections into the posterior one-third.

The thalamic input to this region showed a heterogeneous input to this zone from nuclei in anterior (AM,AV), intralaminar and midline (PCN. Reuniens, CLC, CDC, PF), medial (MDmc,MDpc), ventral (VAmc), lateral (lateral dorsal, lateral posterior and Pulvinar medial) and posterior (Li). The heaviest number of labeled cells were seen in MD, PCN, PUl M and VAmc. With the autoradiographic method, reciprocal connections were noted to the thalamic nuclei with the heaviest numbers of labeled cells.

HRP-positive cells were also seen in the hypothalamus from injections in all three portions of the cortex surrounding the principal sulcus. Cells were seen in the lateral hypothalamic nucleus in all three cases. Cells were only seen in the dorsal medial nucleus, medial mammillary nucleus and tubermammillary nucleus following injections into the midportion.

Projections were also seen throughout the caudate with anterogradely transported proline, lysine and leucine. The fibers were heaviest in the head of the caudate in the dorsomedial portion. Fibers were also seen in the body and tail of the caudate but they were in lower concentration than in the head of the caudate.

The results of this study demonstrate that there are common features to the thalamic and striatal connections to the cortex bordering the principal sulcus. However, only the midportion of this region receives a strong afferent input from the hypothalamus.

622 SELECTIVE HIPPOCAMPAL LESIONS: DIFFERENTIAL EFFECTS ON SPATIAL ACQUISITION AND RETENTION IN RATS. Leonard E. Jarrard. Dept. Psychol., Washington & Lee Univ., Lexington, VA 24450.

Recent research demonstrating different behavioral effects resulting from selective damage to either hippocampal cell fields or hippocampal projections (Jarrard, J. Comp. Physiol. Psychol., 90:1035-1050, 1976), in conjunction with studies implicating the hippocampus in spatial memory (Olton, Walker, and Gage, Brain Res., In Press), prompted the present investigation of the effects of selective hippocampal lesions on acquisition and retention of a complex spatial maze. Rats were divided into two control groups (operated and unoperated) and 5 groups that received bilateral lesions to either the fimbria, dorsal CA1 cell field, alveus, dorsal fornix, or hippocampus (including damage to all cell fields and fimbria). The animals were trained on an eight-arm radial maze. Subjects either underwent training on the spatial maze postoperatively or were trained preoperatively and tested for retention after the operations.

Impaired acquisition and normal retention were found following damage to dorsal CA1 cells and the alveus; both acquisition and retention were impaired in rats with damage limited to fimbria and those with extensive hippocampal lesions. It is suggested that hippocampal projections from the dorsal CA1 cell field to the subiculum play an important role in the acquisition and storage of new spatial information but these connections do not appear necessary for the retrieval of spatial information learned preoperatively. (Supported by NSF Grant BNS 75-18160.)

623 EFFECTS OF COMBINED HIPPOCAMPAL-ENTORHINAL LESIONS ON SPONTANEOUS ALTERNATION, OPEN FIELD ACTIVITY AND SPATIAL MAZE LEARNING IN RATS. Daniel P. Kimble, Dept. of Psychology, U of Oregon, Eugene, OR 97403.

Steward, Cotman, and Lynch (Exp. Brain Res., 1974, 20, 45-66) have shown that following unilateral entorhinal lesions, the normally sparse contralateral entorhinal-hippocampal pathway sprouts and produces a substantial reinnervation of the dentate gyrus on the lesioned side. The present experiment was designed to evaluate the behavioral consequences of severe damage to the ipsilateral entorhinal-hippocampal connections, and the sequence of behavioral changes which might accompany the reinnervation process.

Rats were given unilateral entorhinal lesions combined with contralateral dorsal hippocampal lesions involving the dentate gyrus (HIPP-ENTO, N=11). Control rats were given unilateral lesions of either the entorhinal cortex or the dorsal hippocampus or were unoperated (N=12). Open field activity was measured for 5 min each day on postoperative days 2,4,9 and 14. Spontaneous alternation in an unbaited T-maze was examined on postoperative days 3,5,9,15 and 53. Three different batteries of Rabinovitch-Rosvold maze patterns were used in the Hebb-Williams apparatus. Battery I (mazes 1,5,11) was administered on postoperative days 6,7, and 8. Battery II (mazes 2,6 and 10) was administered on postoperative days 11,12 and 13. Battery III (mazes 4,7,12) was administered on postoperative days 49,50 and 51. Postoperative testing times were chosen to correspond to before, during and after the peak period of reinnervation as reported by Steward, et al.

Spontaneous alternation was reduced to chance levels in the HIPP-ENTO rats on days 3,5 and 10. Partial recovery was seen on day 15 and complete recovery (77% alternation) on day 53. Lesion induced hyperactivity in the open field was seen in the HIPP-ENTO rats on days 2,4 and 9 but was not significantly different from controls on day 14. In contrast to these results, a significant and persistent maze learning deficit was observed. HIPP-ENTO rats made significantly more errors on Battery I (173 vs 82), Battery II (79 vs 48) and Battery III (69 vs 35). Statistical significance was evaluated using a Mann-Whitney U-test ($p = .002$ for each battery). Thus, while there is behavioral recovery of normal levels of spontaneous alternation and open field activity, whatever contralateral reinnervation is taking place in these rats is apparently not capable of supporting recovery of the lesion induced maze learning deficit during the postoperative period under study.

624 RADIO-TELEMETERED ELECTRICAL ACTIVITY FROM AMYGDALA DURING SOCIAL BEHAVIOR IN MONKEY. Arthur Kling, Sid Deutsch* and Horst D. Steklis*. Dept. of Psychiatry and Bioengineering, CMDNJ-Rutgers Medical School, Piscataway, N.J. 08854.

Electrical activity from the amygdala was recorded in 6 monkeys (C. aethiops) during social interactions via a totally subcutaneous radio-telemetry unit with an external turn on-off device.

Relatively artifact-free recordings were obtained from all subjects from a single electrode chronically implanted in the left baso-lateral amygdala. Each subject was studied for 2-3 weeks with varying numbers of conspecifics in a large lexan enclosure with a loop antennae. The electrical activity was recorded on a polygraph and data tape recorder, and later subjected to spectral analysis.

Fourteen behavioral interactions were associated with consistant patterns of amygdala activity. Highest power outputs, especially at the faster frequencies, were associated with active and passive "genital inspection" and "being threatened", followed by "running" and "being chased". Lowest outputs, (except during sleep) were associated with tension reducing behaviors of active and passive grooming.

Additional recordings from multiple brain sites using a multichannel transmitter indicates differential responses during discrete behavioral interactions from other brain sites. Amygdala activity from the latter device support the findings from single channel recordings.

These results suggest that electrical activity from amygdala during social behavior is related to the "affective context" of the interaction rather than the overt behavior.

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625 TWO-RSA CONCEPT SUPPORTED: EFFECTS OF ATROPINE, URETHANE AND SEPTAL STIMULATION. R.C. Kramis* and C.H. Vanderwolf. Dept. Psychology, Univ. Western Ontario, London, Canada.

Electrical stimulation of the diagonal band of Broca (DBB) in 8 chronically implanted rats elicited synchronous slow wave electrical patterns bilaterally in hippocampus during movement and immobility. These elicited patterns 1) appeared similar to spontaneously generated hippocampal rhythmical slow activity (RSA), i.e. theta, but were driven at stimulus train frequencies, and 2) were affected by atropine sulfate and ethyl urethane in the same way as spontaneously generated movement- and immobility-related hippocampal RSA patterns. The data support the contention (Kramis, Vanderwolf, & Bland, Exp. Neurol., 1975, 88, 300-323) that hippocampal RSA is not a unitary phenomenon, but rather is generated by two pharmacologically and behaviorally distinct systems.

In each rat: 1) Appropriate stimulation (e.g. 0.2 msec pulse duration, 4.0 msec inter-pulse interval, 40 msec train duration, 5-12 trains/sec, 1-6 V., 5-60 μa) elicited frequency-specific hippocampal slow waves during movement and immobility. 2) After atropine sulfate injection (50 mg/kg) DBB stimulation was ineffective unless the animal moved, whereupon frequency-specific waves were immediately elicited. Thus the neural system mediating hippocampal slow waves during immobility had apparently been blocked by atropine. 3) Ethyl urethane (0.5 - 1.5 g/kg) produced immobility and prevented elicitation of the movement-associated hippocampal slow waves which remained after atropine injection alone. 4) Ethyl urethane alone allowed DBB elicitation of frequency-specific hippocampal slow waves during the urethane-induced immobility. This indicated that urethane blockade of the hippocampal waves associated with movement after atropine was specific to a movement-related neural system and was not due to a general CNS depression. 5) The hippocampal slow waves elicited during the immobility induced by urethane alone were abolished by atropine sulfate just as were the waves elicited during spontaneous immobility in the undrugged animal.

Similar stimulation of lateral septum (8 rats) produced hippocampal frequency-specific slow waves which were not, however, abolished by the above drug treatments. Perhaps the lateral septal but not the DBB stimulation effects were antidromic. Caudate and globus pallidus stimulation produced no frequency-specific hippocampal patterns although hippocampal RSA occurred when movement was elicited.

626 AUTORADIOGRAPHIC DEMONSTRATION OF HABENULOPEDUNCULAR AFFERENTS TO RAT INTERPEDUNCULAR NUCLEUS. Nicholas J. Lenn and Viviana Wong*. Dept. Neurol.,Pediat.,Carnegie Lab.Embryol.,Sch.Med., Univ. of California, Davis, CA 95616.

In order to further clarify the distribution and electron microscopic identification of interpeduncular nucleus (IPN) afferents from habenula (Lenn, J.Comp.Neur.166:73-100,1976), ^{3}H-leucine was injected into the habenular region. Light and electron microscopic autoradiography was performed after one day's survival.

Following injections which labelled predominantly medial habenula neurons, the habenulopeduncular tract (HPT) was massively positive. After an asymmetrical injection with predominant labelling of one HPT, the ipsolateral half of IPN was more heavily labelled than the contralateral half. There was a horizontal, linear pattern to the silver grain distribution, representing the horizontal axon plexus. HPT was recognized as a distinct tract lateral to IPN at all levels of the nucleus. HPT decreased in size and intensity of label from rostral to caudal as fibers left it to cross IPN.

Electron microscopic autoradiographs were analyzed by determining relative grain densities (RGD: % label over a tissue component divided by % area occupied by that component) in random micrographs of IPN. For 1100 silver grains, the preterminal and terminal axonal RGD was 2.6, and the dendritic RGD, 0.14. The axonal labelling predominated over the horizontal axon plexus, in agreement with previous data and the light microscopic observations. The synaptic labelling was mostly in relation to S synapses, confirming previous degeneration experiments. The crest synapses, also considered habenulopeduncular afferents on the basis of degeneration experiments, were labelled in 95% of the 21 cases observed. The degeneration study had suggested that crest synapses were formed by one axon from each habenula. In the autoradiographs after markedly asymmetrical labelling of medial habenula, 75% of the labelled crest synapses showed labelling of only one of the axonal endings. This again supported a bilateral origin of crest synapses in the normal IPN. The identification and distribution of habenulopeduncular afferents has been detailed, confirming and elaborating results obtained by other methods. (Supported by NIH grants #NS12265, #HD08658. Facilities of the Calif. Primate Res. Ctr. supported by NIH grant #RR00169 were used).

627 VENTRAL GLAND MARKING AND OPEN-FIELD ACTIVITY CHANGES FOLLOWING DISRUPTION OF ACCESSORY, MAIN, OR BOTH OLFACTORY SYSTEMS. Georgia l'Hommedieu-Vitale*. (SPON: E. M. Hull). Dept. Psych., SUNY Buffalo, Buffalo, N. Y. 14226.

Lesions of central and peripheral olfactory structures differentially affect various behaviors, e.g., activity, aggression, and sexual behavior. Central lesions disrupt both the main and accessory olfactory systems. Peripheral lesions are most commonly produced by necrocoagulation following washing the main olfactory epitheleum with a $ZnSO_4$ solution, which does not disrupt the vomeronasal organ (Singh, Tucker, & Hofer, 1976). Thus the effects of central vs. peripheral lesions, which is based on the level of the olfactory system being disrupted, may be reinterpretted as a main vs. accessory olfactory system distinction, based on the disruption of these parallel systems.

Twenty-eight young adult male gerbils were isolated and assigned to four groups of equal size. Four surgical techniques were employed: 1. ablation of the olfactory bulbs:BA, 2. sectioning of the vomeronasal nerves on the medial surface of the olfactory bulbs: VND (after Winans & Powers, 1970), 3. transverse sectioning of the main olfactory bulbs anterior to the accessory olfactory bulbs, without damaging the vomeronasal nerves: BS, and 4. sham control: SH. Open-field activity and social interaction behaviors were assessed on day 14 after surgery. Disruption of either the main or the accessory olfactory system (BS, VND, and BA animals) results in significantly increased activity compared to sham control animals ($p<.01$).

When paired with a standard animal, ventral gland marking of the experimental animal is severely depressed ($p<.01$) only when the accessory olfactory system is disrupted (VND and BA animals). This may reflect the significance of the sensory information that is lost with accessory system disruption, or the significance of the loss of input to those forebrain areas innervated by the accessory system.

628 INVESTIGATION OF THE CHOLINERGIC PATHWAY IN THE NUCLEUS ACCUMBENS. J.E. Marchand*, J.C. Stanley, and J.F. DeFrance. Dept. of Neurobiology and Anatomy, Univ. Tex. Med. Sch. at Houston, Houston, Tex. 77025.

The nucleus accumbens septi (nAcS) might well be considered as a bridge between the limbic and extrapyramidal systems. One of the remarkable features of the nAcS is its intensive staining for acetylcholinesterase (Jacobowitz and Palkovits, 1974; Lewis and Shute, 1967; Srebro, Mellgren and Harkmark, 1976). A study was, therefore, undertaken to investigate the effects of acetylcholine (ACh) and atropine (Atp) in the nAcS.

Microstimulation electrodes were placed in the fimbria-fornix (fi-fo) system and lateral pre-optic (IPO) or anterior medial forebrain bundle (MFB) of acutely prepared rabbits. Field potentials and extracellular unitary potentials were recorded following stimulation.

Field potentials evoked via fimbria-fornix stimulation appeared as complex responses in the nAcS. An early negativity (of monosynaptic origin) was followed by a shallow positivity and there upon by a second negativity. This second negativity was often of low-amplitude and prolonged, but could also become potentiated at certain stimulus frequencies to the point that population action potentials appeared. Unitary recordings confirmed that both negativities represented the action of the same population of cells. ACh and Atp were administered iontophoretically (Atp was also given intravenously in certain experiments). While ACh has a facilitatory effect upon both the monosynaptic and polysynaptically activated field potentials, Atp appeared effective only against the polysynaptic component, blocking it. Stimulation of the IPO or MFB also evoked atropine-sensitive monosynaptic excitatory responses in the nAcS. Therefore, it is possible that fi-fo stimulation activates a IPO acetylcholine pathway to the nAcS that is monosynaptically excitatory. Supported by NSF Grant #S01 RR-05745-03 to JDF.

629 A SENSORY MAPPING OF THE SEPTAL AREA OF THE RAT. L. F. Mercer*and N. R. Remley. Dept. Psych., Texas Christian Univ., Ft. Worth, TX 76129.

Responsiveness of single units in the septal area of male Sprague Dawley rats to sensory stimulation was investigated to determine the distribution and degree of convergence onto the individual units. Unit activity was sampled in six planes transecting the septal area at 0.5mm intervals extending from 0.5mm posterior to 2.0mm anterior to bregma. Test stimuli consisted of light flashes, bursts of white noise, flank and vibrissa contact, quinine and saccharine solutions dropped onto the tongue, and odors of estrous female urine and coffee passed into the nostrils.

Units responding to visual, auditory and olfactory stimulations were found throughout the longitudinal extent of the septal area with relatively uniform distribution between the lateral and medial nuclei and the diagonal band region. Somatosensory responsiveness was found in a somewhat more localized field having medial and ventral prominence and becoming wider in the posterior planes. Gustatory representation was similar to somatosensory distribution with a slightly wider field.

Convergence of inputs was assessed by examining the number of modalities to which each cell responded. There was a tendency for cells in the dorsal aspects of the septal area to show a greater degree of convergence than found in ventral regions.

630 SUBSTANTIA INNOMINATA, SEPTAL AREA AND NUCLEI OF THE DIAGONAL BAND IN THE RHESUS MONKEY: ORGANIZATION OF EFFERENTS AND THEIR ACETYCHOLINESTERASE HISTOCHEMISTRY. Marek-Marsel Mesulam, Gary W. Van Hoesen and Douglas L. Rosene*. Harvard Neurological Unit, Beth Israel Hospital, Boston, Massachusetts 02215.

In 29 rhesus monkeys the hippocampus, the olfactory bulb and diverse neocortical areas were injected with horseradish peroxidase (HRP). The tissue was processed by four distinct histochemical methods: 1) A procedure which yields a blue reaction-product at sites of HRP activity (Mesulam, JHistochemCytochem, '76); 2) A Koelle procedure for acetylcholinesterase (AChE); 3) A Kasa procedure for the indirect visualization of choline acetyltransferase (ChAc) activity; 4) A combined procedure for the simultaneous demonstration of HRP(blue) and AChE(reddish-brown)(Mesulam, ibid.). The focus in this study was limited to the substantia innominata, the septal area and the nuclei of the diagonal band.

Our results show that the traditional subdivisions of the basal forebrain have an orderly organization of efferents such that: 1) Olfactory bulb injections of HRP result in the labeling of neurons predominantly in the nucleus of the horizontal limb of the diagonal band; 2) Hippocampal injections result in the labeling of neurons predominantly in the medial septal area and nucleus of the vertical limb of the diagonal band; 3) Neocortical injections result in labeling of neurons located predominantly in the nucleus basalis of the substantia innominata; 4) Within the nucleus basalis, medially located neurons project predominantly to frontal, cingulate and parietal cortex while the majority of efferents to temporal neocortex originates from neurons located more laterally. None of these topographical arrangements are rigid and various degrees of overlap exist.

We have also shown that the basal forebrain areas under study contain a population of perikarya which are rich in AChE and ChAc content. In fact, the combined AChE-HRP procedure showed that:1) Almost all of the nucleus basalis perikarya which are labeled with HRP after neocortical injections are also AChE-rich; 2) Only a very small proportion of the perikarya which are HRP-labeled after olfactory bulb injections are AChE rich; 3) After hippocampal injections, an intermediate proportion of the HRP-labeled perikarya are also AChE-rich. Hence, the basal forebrain of the rhesus monkey contains a core of AChE-rich perikarya which sends topographically organized efferents to the olfactory bulb, to hippocampus and to widespread neocortical areas. This may constitute a telencephalic extension of the brain-stem reticular formation; it may employ acetylcholine as its transmitter; and it may modulate motivational aspects of behavior. (Supported by NIH grants NS 05403, 09211, 06209 and the Benevolent Foundation of Scottish Rite Freemasonry, Northern Jurisdiction,U.S.A.)

631 FIRING PATTERNS AND BEHAVIORAL CORRELATES OF NEURONS IN ENTORHINAL CORTEX OF FREELY-MOVING RATS. Susan J. Mitchell* and James B. Ranck, Jr. Dept. of Physiol., Downstate Medical Center, SUNY, Brooklyn, N.Y. 11203.

The purpose of this study was to describe the firing of neurons in medial entorhinal cortex (MEC) and the relation of firing rate and pattern to the behavior of the rat. These data allow inferences to be made about the electrophysiology of the system and about information flow in the entorhino-hippocampal segment of the limbic system. Rats studied had bilateral stereotaxically implanted movable microelectrodes aimed at MEC. Additional fixed electrodes were placed to record slow waves in hippocampus and neocortex, and EMG of neck muscles.

One group of neurons was identical to all theta cells of hippocampus and some theta cells of medial septal nucleus (Ranck, J. Exp. Neurol. 41: 461, 1973). These are found in a band (or bands) in layers II or III of MEC, amidst the cells which project to hippocampus. A regular theta rhythm can be recorded in MEC. As the microelectrode moves through MEC the theta rhythm changes its phase 180° with respect to the hippocampal theta rhythm. This phase shift occurs in the outermost layers of MEC suggesting that there is a theta generator there.

Two other groups of neurons with distinct behavioral correlates have been found. One, firing single and complex-spikes (discharges of 2-7 action potentials usually decreasing in amplitude with interspike interval of 1.5-6.0 msec) fired only when a novel object was placed on or near the table. These neurons fired a few times when the object was presented then were off for several minutes. The second group of neurons, firing only single spikes, had distinct spatial characteristics. They fired at their maximum rates (approx. 30/sec) when the rat was at a particular location in the room or when he approached this location.

Another group of MEC neurons fired in bursts of action potentials (intraburst rate 30-50/sec) during SWS. This bursting pattern occurred only when big slow waves were present. Neurons with this pattern are seen in all layers of MEC. Similar patterns of firing have been seen in hippocampus and neocortex by us and others. Some of these neurons fired continuously during awake behaviors at rates either greater than or less than their rates during SWS; others had increased rates of firing during certain other behaviors.
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632 SELECTIVITY IN THE DESTRUCTION OF HIPPOCAMPAL NEURONS BY KAINIC ACID. J. Victor Nadler, Bruce W. Perry* and Carl W. Cotman. (SPON: N.M. Weinberger). Dept. Psychobiol., Univ. Calif., Irvine, CA 92717.

Kainic acid, a potent excitatory analogue of glutamic acid, has previously been shown to destroy neurons in the arcuate nucleus and striatum, while sparing afferent fibers. Presumably, the susceptible neurons are depolarized for a prolonged period, leading to an irreversible ionic imbalance. We have now investigated the excitotoxic action of kainic acid on rat hippocampal neurons.

Adult rats were injected with doses of kainic acid ranging from 0.1 to 3μg, either intraventricularly or directly into the hippocampal formation. Although all hippocampal neurons could be killed by kainic acid, these experiments demonstrated a clear-cut hierarchy of sensitivity to the drug. The most sensitive neurons were those regio inferior pyramidal cells which receive mossy fiber projections (area CA3-4). They were killed within three days by injection of as little as 0.3μg and were replaced by a proliferation of glial cells. At the same time the Fink-Heimer stain revealed, as expected, degenerating fibers and boutons in the commissural, associational and Schaffer collateral terminal zones. Administration of somewhat higher doses was required to destroy the CA1-2 pyramidal cells. Detectable cell loss was seen in this area only when all CA3-4 pyramidal cells had apparently been killed. Hippocampal interneurons were less sensitive than pyramidal cells to kainic acid, and dentate granule cells and subicular cortical cells were killed only when large doses were injected into their immediate vicinity.

Acetylcholinesterase histochemistry was employed to detect any destruction of the cholinergic septohippocampal tract (whose cell bodies were unaffected). In contrast to the massive and rapid hippocampal cell loss, these fibers appeared undamaged, even at the highest doses used. Thus kainic acid evidently affected postsynaptic elements preferentially, in agreement with its actions in other regions. The highly selective toxicity of this drug indicates its utility in studies of hippocampal anatomy and function. (Supported by NSF grant BNS 76-09973 and NIH grant NS 08597).

633 PERIAQUEDUCTAL GRAY LESIONS IN THE RAT: EFFECTS ON PAIN SENSITIVITY, MORPHINE ANALGESIA, VOCALIZATION AND EMOTIONALITY. Gene C. Olson* and John C. Liebeskind. Dept. Psych., UCLA, Los Angeles, CA 90024.

The midbrain periaqueductal gray matter (PAG) has been implicated in a wide variety of specific behaviors. The present study sought to understand the diverse effects of PAG lesions by examining a number of different behaviors in the same animals. Adult male rats underwent electrolytic lesions at various levels of PAG and adjacent tegmentum. Lesions were approximately 1.0 to 2.5 mm in diameter. Animals with PAG lesions at the level of the oculomotor nucleus (rostral lesions) were more active than sham operated animals in the first two minutes in the open field. They also showed an increased baseline latency to paw lick on the hot plate (51.5°C), but only 20% of normal morphine analgesia (10 mg/kg I.P.). Normal vocalization to handling was totally absent in these animals, but they were not mute. Brief squeaks could be elicited by tail shock (1.5 msec pulses at 125 Hz, for 1 second) at 6 times normal threshold. In these animals we were unable to elicit either ultrasonic vocalization or squeaking that outlasted stimulation.

Animals with more caudal PAG lesions at the level of the Dorsal Raphe nucleus did not differ from shams in the open field. They showed a decreased baseline paw lick latency on the hot plate and a slight increase in vocalization. The degree of morphine analgesia was also attenuated in this group, but to a lesser extent than in rostral lesioned animals. Neither group showed long term differences in tail flick latency or body weight, although there were transient feeding deficits accompanying some of the larger rostral lesions. These results support the view that the PAG is importantly involved in emotional expression, and indicate as well that rostral and caudal portions of this structure are functionally distinct.

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634 OLFACTORY AND SEPTAL EVOKED POTENTIALS IN THE DORSAL HIPPOCAMPUS OF AWAKE AND ANESTHETIZED RATS. S. Overmann, R. Bornschein*, D. Woolley* and K. Swanson*. Animal Physiology, University of California, Davis, CA 95616.

Hippocampal (HIP) potentials evoked by stimulation of olfactory cortex (OC), diagonal band of Broca (DB), and lateral septum (LS) were studied in anesthetized rats. Distribution of HIP responses to OC and DB stimulation were surprisingly similar: a low amplitude initially negative response in CA1 and CA2 reversed more ventrally to a high amplitude initially positive response in the dentate gyrus. For both inputs the transition from negative to positive potentials occurred at or slightly below the HIP sulcus. Positive peak latencies were 22 msec for OC stimulation and 41 msec for DB stimulation, with peak negative responses occurring 2-4 msec earlier in each case. The olfactory input to the HIP is via the perforant path. Strong similarities in the distribution of DB-evoked HIP potentials suggest that this input may also use the perforant path. Distribution of HIP potentials to LS stimulation was quite different: an initially negative response in the cortex and superficial HIP lamina was replaced more ventrally in the HIP and dentate gyrus by an initially positive response. The transition from negative to positive potentials was midway between stratum pyramidale and the HIP sulcus. Peak latencies were 16 msec for the positive wave and 22 msec for the negative wave. The HIP response to LS stimulation was believed to be antidromic because of its relatively short latency, stability, and resistance to drug effects, and because the only pathway connecting LS and HIP is efferent to the HIP. In awake chronically implanted rats the single positive wave to OC stimulation observed under anesthesia was replaced by a double positive wave with peak latencies of 20 and 40 msec. The second wave was either abolished or reduced in amplitude and its latency increased by anesthetics, natural sleep, and drugs acting on cholinergic or monoaminergic receptors. Amplitude of the first wave to OC stimulation was doubled by anesthesia, sleep, and drugs acting on noradrenergic receptors. In awake rats the HIP response to DB stimulation was reduced in amplitude and its latency was increased by apomorphine and tremorine, whereas the amplitude was increased by scopolamine, ketamine and sleep. The results indicate that cholinergic and monoaminergic systems are involved in some of the HIP responses to septal and OC stimulation. Furthermore, the second HIP wave to OC stimulation and the DB input to the HIP are reciprocally altered by sleep, some anesthetics, scopolamine, clonidine and phentolamine. This may indicate that septal and olfactory inputs play a reciprocal role in HIP function. (SO supported by NIH Postdoctoral Award # ES05057.)

635 LOSS OF EPISODIC MEMORY IN RATS WITH FORNIX LESIONS: A STUDY ON SINGLE ALTERNATION PERFORMANCE. Michele Pisa* (SPON:D. Bindra). Dept. Psychol. McGill Univ., Montreal, Quebec, Canada H3A 1B1.

Several investigators have suggested an impairment in the mechanisms of extinction to account for the behavioral effects of hippocampus lesions. According to this interpretation earlier reinforced responses compete excessively with conditioning of alternative responses in the presence of the same stimuli.The preempting effect of earlier learning would be ultimately responsible for the amnesic syndrome on account of an abnormal interference between conditioned responses at retrieval. The present experiments tested the alternative possibility that the response disinhibition is secondary to an impairment in recall of episodes or discrete events.

Rats with a sham operation or with a lesion in the fornical connections of the hippocampus were sequentially tested in different versions of a lever-press single alternation task, whose correct performance requires inhibition of the more recent choice. The experimental rats were initially found impaired in acquisition of alternation between closely spaced bars. After increasing the distinctiveness of the alternatives, by placing the levers at the ends of a long alley with the food well midway between them, the lesioned animals immediately learned to alternate as efficiently as the controls. Their performance remained similar to that of the controls when they were subsequently tested for alternation in a T-maze, with the levers in the horizontal arms and the food well in the vertical arm. However, the rats with lesions were again impaired when retested in the original condition with adjacent bars. Also, the interpolation either of baffles or of a 10 sec delay in the vertical arm, both of which resulted in multiple turning responses between choices, severely disrupted the alternation performance of the rats with lesions in the T-maze. Both in the latter cases and in the condition with adjacent bars the experimental rats did not choose randomly,rather they adopted position "hypotheses", alternately perseverating on one or the other choice.

The results indicate that response perseveration, far from being a necessary consequence of hippocampal damage, depends on an inability to recall a discrete event after similar intervening events degrade, through interference,short-term memory of the episode. Being unable to recall the cueing event after distraction, the fornix-lesioned animals attempted to cope with the task by relying on a different source of information, that is the difference between the alternatives in associative strength.

The findings conform to the hypothesis that subjects with hippocampal damage can adequately learn and recall habits, that is reinforced associations, but are severely impaired in retrieval of episodes.

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636 EVIDENCE FOR A SECOND HIPPOCAMPAL-DIENCEPHALIC PHYSIOLOGICAL PATHWAY COMPARABLE TO THE FORNIX SYSTEM--A UNIT STUDY IN THE AWAKE MONKEY. Charles E. Poletti* and Manit Sujatanond* (SPON: Wm. H. Sweet). Massachusetts General Hospital, Boston, MA 02114

In a preceding study in the intact monkey, neuronal firing in response to hippocampal volleys and after-discharges was studied in the hypothalamus, preoptic region, and basal forebrain using extracellular microelectrode techniques. Changes in firing patterns were observed in 22% of 666 units studied. In basal forebrain structures 34% (60) of 177 units responded; in the preoptic region 30% (30) of 99; and in the hypothalamus 14% (56) of 390 units. Response latencies in all three regions were found as short as 10 msecs. This preceding study raised the probability of a hippocampal efferent influence on basal diencephalic structures mediated independent of the fornix system.

The current study tested for a hippocampal influence on basal diencephalic neuronal firing in three chronic awake monkeys with complete bilateral lesions of the fornix system. Single or multiple shock stimulation to chronic bipolar electrodes implanted in the anterior hippocampus significantly altered the firing pattern of 13% (80) of 619 units studied. Changes in firing patterns were observed in 18% (32/174) of units in basal forebrain structures (septal nuclei, gyrus rectus and olfactory tubercle); 5% (3/63) in preoptic areas; and 12% (45/382) in the hypothalamus. The three structures with the highest percentage of responses were the bed nucleus of the stria terminalis (22%), ventromedial nucleus of the hypothalamus (19%) and nucleus accumbens (18%). Minimum initial latencies were 22 msecs. in basal forebrain areas, 25 msecs. in preoptic areas and 20 msecs. in the hypothalamus. Of 80 responding units, 61 (76%) were initially excited, 19 (24%) inhibited. The unit responses were characteristically excitatory with small initial latency variability, and a high firing index to low threshold single shock stimulation.

These combined results establish a second major efferent pathway from the anterior hippocampus to hypothalamic, preoptic and basal forebrain structures comparable in physiological influence to the fornix system. There is evidence that the non-fornix and fornix hippocampal efferent pathways exert a contrasting influence on neurons in the ventromedial nucleus of the hypothalamus.

637 DRUG SENSITIZATION AND ELECTRICAL KINDLING. Robert M. Post, Kathleen M. Squillace,* Willard Sass,* and Agu Pert. Sections on Psychobiology and Biochemistry, Adult Psychiatry Branch, NIMH, Bethesda, Md., 20014. (SPON. R. Wyatt)

A series of studies were performed to examine the interaction of electrical and "pharmacological" kindling paradigms. In Study I, 35 rats were implanted with unilateral amygdala electrodes and pretreated with 10 days of no handling (n=11), daily saline (n=12), or daily cocaine injections (40 mg/kg, i.p., n=12). Electrode placements were verified to be in the amygdala histologically except in one animal whose electrode was in the internal capsule and still showed a normal pattern of kindling. Daily cocaine, which produces increases in sensitivity to motor-activating and stereotypic effects, did not alter the rate of electrical kindling although the saline injected (stressed) animals had a significantly longer onset to first kindled seizure than the nonhandled controls. The effect of lidocaine (40 mg/kg i.p.) pretreatment for 21 days on the rate of subsequent amygdala kindling will be reported. In experiment II, 38 animals were implanted with unilateral amygdala electrodes; 20 were sham stimulated, while 18 were stimulated at 200 μA, 50 Hz, for 500 milliseconds once daily. Following 21 days of stimulation, experimental and control animals were challenged following 3 day intervals with cocaine (10 mg/kg), apomorphine (0.2 mg/kg), cocaine (40 mg/kg), apomorphine (4 mg/kg) and lidocaine (60 mg/kg). At the higher doses of cocaine and apomorphine, the kindled animals had significantly decreased cocaine-induced vertical activity and increased apomorphine vertical activity ($p < .01$). 14 of 16 kindled animals had lidocaine-induced convulsions compared to only 4 of 17 controls (χ^2, $p < .001$). In study III, 19 animals were treated with oral lithium (0.6 meq/15 gms of lab chow) beginning 7 days prior to amygdala kindling and continuing during 21 days of electrical stimulations. Regardless of pretreatment in Studies I-III, approximately 40% of kindled animals showed marked oscillations in after-discharge durations; these animals displayed cycle lengths varying from 2 to 5 days. Lithium did not alter the rate of kindling or the emergence of marked oscillations in after-discharge durations. The results of Study IV examining the effect of naloxone and morphine on electrical kindling of the amygdala will also be presented.

These data indicate that there are important and complex interactions between "pharmacological" and electrical kindling paradigms. Kindling may represent a tool for producing long-lasting alterations in responsivity of selective neural substrates valuable in dissecting biobehavioral relationships.

638 ELECTROPHYSIOLOGICAL ANALYSIS OF EVOKED ACTIVITY IN THE MAMMILLARY COMPLEX OF THE CAT. Harvey Reisine*. (SPON: A. Rudell) Dept. of Physiol., SUNY, Downstate Medical Center, Brooklyn, N.Y. 11203.

Cats were anesthetized with sodium pentobarbital and, in some, the fornix was exposed ipsilateral to the recording site. Bipolar stimulating electrodes were placed according to stereotaxic coordinates in the mammillothalamic tract (MTT) and were placed visually in the dorsal fornix (DFX). Stimuli were square wave pulses, 0.02 to 0.05 msec in duration and 0.1 to 4.0 mA in intensity. Spontaneous and evoked activity was recorded with glass insulated tungsten microelectrodes (10 to 30 μ tip protrusion).

Antidromic labelling of projection neurons in the mammillary complex assisted in identifying the recording site during an experiment. Lesions made at the bottom of a penetration were observed in histological sections and verified electrode positions. Properties of 57 mammillary units, evoked by MTT stimulation, included: 1) all or nothing response at a critical stimulus intensity, 2) unit responses varied in latency less than 0.3 msec, 3) each unit response followed a stimulus train of 200 Hz, 4) the absolute refractory periods of these unit responses, determined by paired pulse stimulation, was 0.5 to 2.6 msec and 5) collision was observed when the spontaneous activity of the unit triggered a stimulus with an appropriate delay. The latencies of unit responses ($\leq$ 4 msec) correspond to the earliest prominent (0.5-2.3 msec) negative component (1-3 msec latency) of the field potential recorded in the mammillary complex following stimulation of the MTT.

Stimulation of the DFX at 1 Hz results in a multi-component field potential recorded in the mammillary complex, including an early negative component (4-6 msec latency) which follows a 100 Hz stimulus train. A negative component is observed following stimulation of the post-commissural fornix with a shorter latency (2.5-3.5 msec) which occludes the negative component evoked by DFX stimulation, implying that a common set of afferents is being stimulated. Conduction velocity of the fornix fibers is estimated at 3.5 m/sec. Unit responses following stimulation of the DFX have latencies of 9 to 20 msec and follow a stimulus train no greater than 20 Hz implying that they are postsynaptic. When the stimulating electrodes are moved deeper into the DFX, the amplitude of the negative component is diminished and a different, positive-negative, wave is recorded, the positive peak having a latency ranging from 30 to 35 msec. This wave does not follow a stimulus train greater than 5 Hz implying a postsynaptic origin. Both evoked potentials from DFX stimulation were unaffected by destruction of the rostral pole of the hippocampus, i.e., posterior to the stimulating electrodes. (Aided by NS0577303).

639 INFLUENCE OF LATERAL SEPTUM (LS) STIMULATION ON THE EXCITABILITY OF MEDIOBASAL HYPOTHALAMIC (MBH) NEURONS IN THE RAT. Leo P. Renaud, H.W. Blume*, Q.J. Pittman, R.E. Kearney* and B.W. MacKenzie*. Div. of Neurology, Montreal General Hospital, Montreal H3G 1A4, Canada

Previous electrophysiological studies on MBH neurons of the rat have demonstrated that many of these cells, in particular the neurons in the ventromedial nucleus, have afferent and efferent connections with extrahypothalamic areas, e.g. the amygdala, periaqueductal gray (PAG), midline thalamic nuclei and medial preoptic area (Brain Res. 93: 145-151, 1975; J. Physiol. 260: 237-252, 1976; J.Physiol. 264: 541-564, 1977).

In pentobarbital anaesthetized male Sprague Dawley rats, further studies were conducted on 267 MBH neurons to examine their excitability during single 1 Hz LS stimulation. A PDP 11/40 computer was used to analyze spike discharge probability patterns after LS stimulation, to compare these responses to those elicited by stimulation in the amygdala and PAG, and to examine the topography of the tested neurons. 46% of neurons (n = 124) tested with LS stimulation were unresponsive. Of the remainder, only 3 cells displayed antidromic invasion (mean latency 6.7 msec $\pm$ 4.5 S.D.) indicating that very few MBH neurons project to LS, in contrast with more than 18% of MBH cells that project to the medial preoptic-anterior hypothalamic area. 40% of neurons (n = 109) displayed orthodromic excitation (latency range 4-52 msec; mean 16.7 $\pm$ 8.4 S.D.) while 12% of neurons (n = 31) displayed a depression of activity (latency range 4-40 msec; mean 19.0 $\pm$ 11.2 S.D.) after LS stimulation. Most of the responsive neurons were localized in the ventromedial and paraventricular nuclei, and the anterior periventricular region. Approximately 50% of responsive cells in the ventromedial nucleus also displayed orthodromic activation or depression after amygdala stimulation, indicating a significant convergence of afferent pathways. In contrast, few LS responsive neurons were influenced by PAG stimuli. These studies indicate that both LS and amygdala are important afferent sources of information for neurons in the ventromedial nucleus.

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640 EFFECTS OF MORPHINE AND DIAZEPAM ON SPONTANEOUS NEURON ACTIVITY IN THE LIMBIC SYSTEM OF THE CAT. J.H. Robinson* and S.C. Wang* (SPON: Martin C. Wallenstein). Dept. of Pharmacology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

The effects of morphine and diazepam (Valium) were studied on spontaneous unit activity in locally anesthetized, immobilized cats which had been surgically prepared under halothane anesthesia. Glass-coated, platinum-iridium microelectrodes were used to record single neurons in the cingulate gyrus, septum and the lateral hypothalamic area. Morphine sulfate (1.0-2.0 mg/kg i.v.) augmented the spontaneous discharge rates of the majority (64%) of the neurons tested (n=33). This morphine induced increase was as much as 500% in some cases. In contrast, diazepam (0.1-0.2 mg/kg i.v.) reduced the spontaneous discharge rates as well as the augmented firing rates induced by morphine injection in 85% of the neurons tested (n=26). At times, administration of diazepam attenuated the morphine augmented firing rates below pre-morphine control levels. These data are qualitatively similar to that reported on the amygdala and hippocampal formation (Chou and Wang, Fed. Proc., Vol. 35, 3, 269, 1976). Finally, administration of two neuroleptic agents, haloperidol (0.5-0.1 mg/kg i.v.) and perphenazine (0.1-0.3 mg/kg i.v.) had no significant or consistent effect on morphine induced firing rates. These findings, along with data reported on the amygdala and hippocampus, suggest that, in cats, the entire limbic system may play an important role in morphine-induced behavioral responses and may also represent the site of action for the tranquilizer diazepam. (Supported by NIH Grants #HL 12738, #NS 05173 and #NS 00031.)

641 EFFECTS OF ACETYLCHOLINE, SUBSTANCE P AND STIMULATION OF HABENULAR NUCLEI ON RAT INTERPEDUNCULAR NEURONAL ACTIVITY. B.R. Sastry* (SPON: J.W. Phillis). Department of Physiology, College of Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N OWO.

The high levels of choline acetyltransferase and acetylcholinesterase activities in the interpeduncular nucleus (IPN) as well as the reportedly reduced activity of the former enzyme following the destruction of the rat brain habenula suggest that the habenulo-interpeduncular pathway (HIP) may be cholinergic. The levels of substance P (SP) in the IPN also decrease after a lesion in the habenular nuclei. Therefore, both acetylcholine (ACh) and SP may be involved in the HIP-induced effects on IPN cells. In male Sprague Dawley rats anaesthetized with a mixture of methoxyflurane, nitrous oxide and oxygen, the activity of single IPN cells was recorded through the central barrel (2 M NaCl) of 7-barrel micropipettes. The outer barrels contained drugs that were iontophoretically applied and 2 M NaCl for current controls. Many neurones in the dorsolateral IPN exhibited rhythmic activity. These cells fired at 15-100 Hz for 10-30 sec duration and this activity was interrupted by silent periods of 20-35 sec. Other neurones in the dorsal and ventral IPN discharged single spikes or small bursts of spikes at irregular intervals. Stimulation in the habenular nuclei (1 Hz) produced the following effects on the IPN neurones: 1) a short latency (< 5 msec) depression of brief duration (< 100 msec) occasionally followed by a weak facilitation; 2) a short latency excitation (about 5 msec) followed by a prolonged weak depression; 3) a long latency (> 20 msec) excitation of prolonged duration (200-500 msec); or 4) a long latency depression (> 20 msec). The IPN cells that were excited by the habenular stimulation were also activated by ACh and SP. In the majority of the cases atropine antagonized the facilitatory effect (long latency facilitation) of habenular stimulation and the excitation by ACh. The effects of SP were not substantially altered by atropine. Lioresal antagonized the long latency excitation produced by the activation of HIP and SP excitation but it also antagonized ACh. γ-Aminobutyric acid (GABA) depressed the IPN cells and bicuculline antagonized this depression. The inhibition of the IPN neurones by activation of HIP was unaffected by bicuculline. The rhythmicity of the dorsolateral IPN cell firing was unaltered by stimulation of the habenula, or by the application of ACh, SP, atropine and Lioresal (see also Lake, Exp. Neurol. 41, 1973). The results of this investigation indicate that 1) ACh and SP in the HIP may be involved in mediating the long latency excitation of IPN cells and 2) GABA is not involved in the inhibition of IPN cells produced by stimulation of the habenular nuclei.

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642 PARALLELISM BETWEEN THE EFFECT OF LESIONS OF THE CORTICO-MEDIAL OR BASO-LATERAL AMYGDALA ON BEHAVIORAL AND PLASMA CORTICOSTERONE RESPONSE TO STRESS. Jo A. Seggie and John Chambers*. Neuroendocrine Research Section, Clarke Institute of Psychiatry, 250 College St., Toronto, Ontario M5T 1R8.

It has been suggested that the amygdala may be involved in regulation of adrenal corticosteroid secretion. The present study was undertaken to determine the effect of lesions of the cortico-medial or baso-lateral amygdala on the adrenal corticosteroid response to stress. Data presented elsewhere have indicated that neither of these lesions has any effect on normal resting levels or on 24-hour variations in plasma corticosterone. The baso-lateral amygdala lesion used in the present study produced an increase in behavioral reactivity as measured by resistance to capture, freezing and startle response while the cortico-medial amygdala lesion had no effect on these behaviors.

Adult male rats were housed in individual cages under a light cycle of 12 hours light/12 hours dark. Separate groups of normal, sham-operated or amygdala-lesioned rats were subjected to one of two stressors at the same time of day and sacrificed by decapitation 0, 5, 10 or 15 minutes after the end of stress. Trunk blood was collected for hormone assay. A low level of stress was imposed by picking the animal up for 5 seconds while a higher level of stress was imposed by placing the animal into a novel environment for 3 minutes. In the first study, lesions were placed bilaterally in the baso-lateral amygdala while in the second study the lesion group sustained cortico-medial amygdala lesions.

Cortico-medial amygdala lesions had no effect on the pattern of plasma corticosterone responses to either of the stressors. The baso-lateral amygdala groups, however, exhibited a corticosterone response to stress that was of shorter latency and greater magnitude than the non-lesioned controls. Thus, the 2 types of amygdala lesions had parallel effects on behavior and adrenal stress response without affecting resting corticosterone levels. Baso-lateral amygdala lesions potentiated both the behavioral and adrenal responses to stress while cortico-medial amygdala lesions had no effect on either of these variables. The former finding is reminiscent of the effects of septal lesions which potentiate both behavioral and hormonal indices in response to stress in rats without affecting resting hormone levels (Biological Psychiatry 11(5): 583-597, 1976).

This study was supported by the Ontario Mental Health Foundation Grant #729-76/78. Dr. Jo A. Seggie is an O.M.H.F. Scholar.

643 EFFERENT CONNECTIONS OF THE SUBSTANTIA INNOMINATA IN THE CAT. Allan Siegel and Raymond Troiano*. Dept. of Neuroscience, New Jersey Medical School, Newark, New Jersey, 07103.

The efferent connections of the substantia innominata in the cat were studied utilizing the technique of ^{3}H-amino acid radioautography. Injections of ^{3}H-leucine (0.2-0.3 μL., 20 μCi/μL) were systematically placed throughout all levels of the substantia innominata and adjacent structures. Survival times varied from 1-2 days. Our results indicate that cells situated in the most lateral portions of the substantia innominata project heavily to the medial and central amygdaloid nuclei via two routes -- a direct ventral pathway and the more circuitous stria terminalis. These neurons also project caudally to the bed nucleus of the inferior thalamic peduncle, lateral hypothalamus, and ventral tegmental area. Rostral projections of this region were traced to the olfactory tubercle, nucleus accumbens and bed nucleus of the stria terminalis. The efferent connections of more medial and caudal portions of the substantia innominata resemble the projections of the lateral hypothalamus and include such targets as the lateral hypothalamus, ventral tegmental area, lateral habenular nucleus and septal area. Therefore, this component of the substantia innominata may be viewed as a rostral extention of the preoptico-hypothalamic continuum. Whenever the large, angular, multipolar cells of the basal nucleus of Meynert were labeled, axons were observed projecting towards cortical areas. When the labeled cells within this group were situated medially, axons projected around the genu of the corpus callosum into the prefrontal cortex and anterior limbic area. When the labeled cells were situated laterally, axons projected to the pyriform and prepyriform cortices. Additionally, other fibers also appeared to extend into temporal and parietal neocortical regions from this site.

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644 REFRACTORY PERIOD ESTIMATES OF NEURONS MEDIATING THE APPETATIVE AND AVERSIVE EFFECTS OF ELECTRICAL STIMULATION OF THE LATERAL HYPOTHALAMUS IN THE RAT. Ronald W. Skelton* and Peter Shizgal, Dept. of Psych., Concordia Univ., Montréal, Québec Canada.

Does escape from rewarding Lateral Hypothalamic stimulation (LHS) reflect the direct excitation of neural elements other than those mediating the rewarding effect? Deutsch and Albertson (Behav. Biol. 11, 1974) addressed this question by behaviourally estimating refractory periods (RP's) of the neural elements subserving escape responses and self-stimulation. Yeomans (Physiol. Behav. 15, 1974) criticized their scaling methods suggesting an alternate procedure used in our partial replication of their work.

Rats were trained to press one lever to turn on and another to turn off trains of single or paired pulses. The frequency thresholds for on- and off-responding were determined for 9 intrapulse intervals (IPI) and compared to frequency thresholds for single pulses. If the ratios of frequency thresholds thus derived estimate the relative effectiveness of the second pulse of each pair (E_2), then changes in E_2 with IPI should reflect the RP of directly stimulated neural elements.

The increases in E_2 over IPI of .6-1.2 msec were similar for on- and off-responses and so indicated similar refractory periods for the neural elements mediating the two behaviours. However, in some rats, E_2's for the different behaviours were different at IPI = .2 msec. Yeomans (ibid.) has argued that E_2 at this IPI reflects local potential summation in elements too distant from the electrode to be fired by the first pulse of each pair. This implication of differing spatial distributions of neural elements involved in on- and off-responses is consistent with previous reports of different current thresholds as well as our own findings of differing frequency-intensity functions. These results are best explained by assuming that the electrode directly stimulates two functionally distinct neural populations. Thus, although our results are similar to those of Deutsch and Albertson, we disagree with their RP values and their conclusion that a single population of neural elements is responsible for both responses.

645 EFFECTS OF SCOPOLAMINE AND ATROPINE ON HIPPOCAMPAL EEG IN THE CAT. Charlotte S. Smylie*, Richard B. Lenig* and Christopher J. Frederickson (Spon: R. D. Stillman). Program in Psych., Univ. of Texas at Dallas, Box 688, Richardson, TX 75080

Four cats with chronic EEG electrodes yielding high-amplitude (350-750 uv) Rhythmical Slow Activity (RSA) from the dorsal hippocampus were trained to wait immobile till a door opened, then walk through an alley to food. All animals showed the following EEG patterns during performance: waiting was generally accompanied by RSA (4-7 Hz; 350-700 μv) with intermittant episodes of Small Irregular Activity (SIA) (5-30 Hz; 200-400 μv) and occasional rare bursts of Large Irregular Activity (2-30 Hz; 350-600 μv); walking was accompanied by continuous RSA (5-8 Hz; 350-650 μv); lapping the food was accompanied by mixtures of slow, small RSA (3-7 Hz; 200-400 μv) and SIA.

Comparatively small doses of scopolamine hydrobromide (.05 mg/kg) or atropine sulfate (1.0 mg/kg) (i.p.) virtually abolished the RSA during immobile waiting and food lapping. After injection, waiting was accompanied by LIA (up to 900 μv) with occasional periods of SIA, and lapping was accompanied by roughly equal amounts of LIA and SIA. The same drug doses did not, however, entirely block the RSA which accompanied walking. RSA was present at least sometimes during walking in all drug tests, and in most cases RSA was the predominant pattern during walking. Still, some episodes of walking without RSA did occur in every drug test.

Large doses of scopolamine (0.5 & 1.0 mg/kg) and atropine (10 and 20 mg/kg) severely depressed spontaneous walking and all but eliminated food lapping. During immobility, LIA was essentially continuous except for occasional episodes of SIA. During walking, LIA and SIA were the predominant patterns, SIA often marking the initiation of movement and LIA accompanying continued walking. However, in all cases, RSA was also occasionally recorded during walking. No consistent behavioral differences between the walking accompanied by RSA and that accompanied by SIA or LIA were apparent, although RSA was generally more likely when walking was provoked by handling than when walking began spontaneously.

The results suggest (1) that the RSA which accompanies immobility or food lapping in the cat is more sensitive to anticholinergic blockade than that which accompanies walking, and, (2) that high doses of antocholinergics can block some, but not all, of the RSA which accompanies walking in the cat.

646 EVIDENCE FOR CHANGES IN CNS RESPONSIVENESS FOLLOWING HYPOTHALAMIC STIMULATION DURING HIBERNATION IN GROUND SQUIRRELS (CITELLUS LATERALIS). T. L. Stanton* and A. L. Beckman. Dept. Physiol., Sch. Med., Univ. of Pa., Philadelphia, Pa. 19104.

Previous work has demonstrated that a given dose of acetylcholine (ACh) microinjected into the midbrain reticular formation (MRF) produced transient increases in body temperature (T_b) that progressively increased in magnitude as time in the bout elapsed. The present study examined whether a similar progressive change in CNS responsiveness occurs following ACh stimulation of the preoptic/anterior hypothalamus (PO/AH), which produces arousal from hibernation. A bilateral microinjection of ACh (20, 50, or 100μg) was given during different portions of the hibernation bout via chronically implanted cannulae. Metabolic rate and T_b were continuously recorded during the tests. The results showed a rapid change in responsiveness occurring in the early portions of the bout. No change in T_b was evident in the 1st quarter, although a small increase in oxygen consumption was observed following 100μg of ACh. In the 2nd quarter, increases of 0.10°C to 5.2°C were observed. After 50% of the bout, it was rare to observe any increase in T_b that did not culminate in full arousal from hibernation. The results indicate that whereas a change in CNS responsiveness during hibernation is evident following stimulation of the PO/AH, it differs in character from the more progressive change in responsiveness observed throughout the bout following MRF stimulation. (Supported by USPHS grant NS10597.)

647 BASKET AXON PROJECTIONS IN THE DENTATE GYRUS. R. G. Struble*, N. L. Desmond*, W. B. Levy and A. H. Riesen (SPON: P. D. Wilson). Dept. of Psych, UC-Riverside, Riverside, CA 92521.

The detailed descriptions of the hippocampus by Ramon y Cajal, Lorente de Nó, and others have been of significant aid in directing and interpreting neurophysiological investigations of this structure. As a prime example, Andersen and colleagues (In The Hippocampus, vol. I, 1975) have pinpointed the hippocampal basket cell as the source of the powerful, local recurrent inhibition associated with granule and pyramidal cell activation. However, the report by Andersen and Lømo (In Basic Mechanisms of the Epilepsies, 1969) describing an inhibition in the dentate gyrus oriented along the longitudinal, i.e., septo-temporal, axis of the hippocampus is unexpected if not incompatible with the reported projections of basket cells.

Using the rapid-Golgi method on the dissected, linearized rat hippocampus, this study extends the classical findings of Ramon y Cajal and Lorente de Nó. In particular, this report provides new data regarding the vector of basket cell projections relative to the hippocampal lamellar structure.

Two classes of basket cells were observed in the dentate gyrus. Basket nerve endings were found which were traced back to axons originating in the hilar region of the hippocampus. Such projections were limited to 2-3 transverse sections. As such, these basket cells are similar to those described by Lorente de Nó.

Distinctive from the basket cells with hilar-localized somata are those located in the dorsal leaf of the dentate gyrus. These pyramidal-shaped cells are located within and just subjacent to the granule cell layer. From serial section observations, it is apparent that these cells project some distance in the longitudinal axis. The mean (±SEM) septo-temporal trajectory length was 1045 (±67) μm, while the trajectory within a section, i.e., transverse to the septo-temporal axis, was 387 (±34) μm (n=11).

Although Cajal did describe these basket cells, he apparently did not appreciate the extent of the axonal projections, possibly due to the difficulty in visualizing this longitudinal projection in the normal, unstraightened hippocampus and because of the lack of emphasis on the lamellar organization of the hippocampus until Lorente de Nó. Closer analysis of the terminal ramifications of the basket axons showed that not all areas within the projection field of one basket cell axon were contacted by terminal ramifications of the observed basket axon. Rather, there was a checkerboard-like distribution of the axon plexus with large areas within the projection field free of the endings of a single basket axon. Thus, these basket cells would quite likely be the origin of the longitudinal inhibition previously described in the dentate gyrus. (Supported by PHS grant HD 10401-13A1 to A. H. Riesen and NSF grant BNS 75-18089 to W. B. Levy)

648 THE TOPOGRAPHIC DISTRIBUTION OF SOME INTRINSIC HIPPOCAMPAL CONNECTIONS IN THE RAT. L.W. Swanson, J.M. Wyss and W.M. Cowan. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

The organization of the intra-hippocampal connections of the dentate gyrus and Ammon's horn (fields CA1-4) have been examined with the autoradiographic, HRP, and Timm's methods, the results being plotted on an unfolded map of the hippocampal formation prepared from a large scale model (1:82). Mossy fibers from cells in the inner blade, from the crest region, and from the outer blade of the dentate gyrus, all project for approximately the same distance and in the same general direction through both the infra- and supra-pyramidal bundles. These fibers end adjacent to a narrow, mossy fiber-free, transition zone of the regio inferior, near the CA2/CA3 boundary. The distribution of the mossy fiber pathways depends, however, on its septo-temporal origin. Thus in the septal one-third of field CA3, the mossy fibers follow an "L-shaped" path, crossing transversely and then longitudinally in a temporal direction parallel to the transition zone. At more temporal levels the mossy fibers are essentially transversely disposed.

Field CA4 appears to project bilaterally to the dentate gyrus, to fields CA1, CA3 and CA4 of Ammon's horn, and to the subiculum. Following small ^{3}H-proline injections involving cells in the septal two-thirds of field CA4, without involvement of field CA3c, transported label can be found over a wide area involving the septal one-half to two-thirds of the terminal field. Neurons in the temporal part of field CA4 have a more restricted bilateral projection to the temporal one-fourth of the dentate gyrus, Ammon's horn and subiculum. Similarly, small ^{3}H-proline injections into the septal one-half of field CA3 label extensive bilateral parts of the septal two-thirds of fields CA1 and CA3, and a limited part of the subiculum adjacent to CA1. More temporal injections also label a septally-directed pathway which seems to correspond to Lorente de Nó's "longitudinal association" bundle. The overall extent of the labeled terminal field following injections of either field CA3 or CA4 was quite similar on the two sides of the brain, but that on the ipsilateral side was invariably slightly larger. Field CA1 has been found to project to adjacent parts of the ipsilateral subiculum, with a septo-temporal organization similar to that of the mossy fibers. The results emphasize the highly organized interrelationship between Ammon's horn and the subiculum and also indicate that a small region of the dentate gyrus can widely influence other parts of the dentate gyrus, Ammon's horn, and the subiculum, of both sides.

649 AN EFFECT OF THE LATERAL SEPTUM ON LATENT INHIBITION. A.W.Toga,* H.Burton,* D.G. Davenport,* and S. Horenstein. Depts. of Psychology and Neurology, Saint Louis University, Saint Louis, MO 63104.

Latent inhibition was attenuated in a series of rats which underwent bilateral destruction of the lateral nucleus of the septum. The phenomenon of latent inhibition refers to a lower rate of responding in the presence of a conditioned stimulus that was previously presented without reinforcement. Prior to surgery all animals were trained by appetitive conditioning to press a bar upon illumination of a cue (S^+) but not to the house light. Neither was lit during the intertrial intervals (ITI). The criterion for entering the second phase of the experiment was 80% response to S^+. Upon reaching this level subjects were divided into 4 groups matched for response rates. Two of the groups were assigned to the brain lesion and two to the sham condition. Bilateral ablation of the lateral septum was accomplished stereotactically. The sham controls underwent similar surgery except that the electrodes were lowered to just above the septum. Half each of the operated and control groups were assigned to a preexposure phase in which they received 180 presentations of a 1000 Hz tone within 60 minutes. The other animals sat in an identical training box without tone for 1 hour. Immediately following preexposure all animals were subjected to the first of 4 daily successive discrimination training sessions. S^+ now consisted of a simultaneous 5 sec exposure to the tone, house and cue lights, and S^- the latter two alone. After testing the animals were killed by formolsaline infusion and the brains removed for verification. Latent inhibition or a delay in the initial learning following preexposure was less pronounced in the lesioned animals. Additionally, the septal (lesioned) animals responded more often in the presence of S^-. Finally, these animals had lower learning rates. The perseveration resulting from these lesions resembles that following more extensive septal ablation. A response-perseveration model to explain these results seems appropriate since the septal animals responded in the presence of S^- and had a lower learning rate. One may conclude that destruction of the lateral septum releases behavior from those mechanisms which normally link it to appropriate stimuli.

650 SPECTRAL STUDIES ON 40 Hz, RHINENCEPHALIC RHYTHM, IN THE ELECTROENCEPHALOGRAM (EEG). C. C. Turbes, J. M. Simard* and G. T. Schneider*. Creighton University School of Medicine, Omaha, NE 68178 U.S.A.

These studies were on eight cats with chronic implanted electrodes in the amygdala, nucleus accumbens septi, left and right frontal, parietal, temporal and occipital cerebral cortical areas. Recordings were made with Grass Model #8-10 electroencephalograph. Three channels were recorded on an F.M. tape recorder for further analysis of data. The data was processed with a Varian V-72 minicomputer. Channels were sampled alternately at 2.5 ms and 120 data segments for each channel of 2.56 seconds duration each. This 2.56 seconds of data was transformed using a Fast Fourier Transform algorithm to give a power spectral estimate.

The 40 Hz rhythm, in the amygdala, was most apparent in the alert, hungry, oriented and searching cat. All neocortical areas showed 40 Hz activity during these behavior states. Amphetamine enhanced the 40 Hz activity in the amygdala and neocortical areas. There was also a shift to 48 Hz to 50 Hz in the amygdala during the action of d-amphetamine. Depressants, barbiturates, decreased and eliminated 40 Hz activity at amygdala and cerebral cortex.

651 A POSSIBLE PROJECTION TO THE INFERIOR COLLICULUS FROM THE VENTRAL MESENCEPHALIC TEGMENTUM. S. R. Vincent*, T. Hattori and E. G. McGeer (SPON: A. Jakubovic). Div. of Neurol. Sci., Dept. of Psych., Fac. of Med., Univ. of British Columbia, Vancouver, B. C. V6T 1W5, Canada.

Using light and electron microscopic techniques, evidence for a projection from the ventral mesencephalic tegmentum to the inferior colliculus has been obtained. Rats were given unilateral stereotaxic injections of 0.1 µl of 60% horseradish peroxidase (HRP) into the lateral portion of the inferior colliculus. One day later they were perfused and processed for light microscopic examination. HRP-labelled cells of about 20 micron diameter were observed in the medial geniculate and in the A-10 region of the ventral mesencephalic tegmentum ipsilateral to the injection site.

Following the unilateral injection of 0.25 µl of tritiated leucine (10 µCi/µl) into the A-10 area, transport of label to the ventro-medial portion of the inferior colliculus was observed by light microscope autoradiography. The synaptic morphology of the terminals of this projection was explored further by electron microscopy following lesions of the A-10 region. Rats received unilateral stereotaxic electrolytic lesions (1 mA for 40 sec) of the A-10 area and two to three days later were perfused and prepared for electron microscopic examination. Degenerating boutons of about one micron diameter were observed in the ipsilateral inferior colliculus. Both symmetric and asymmetric axodendritic synapses were observed to degenerate. Some myelinated fibres were also seen to be degenerating in this region.

Although the A-10 area is known to contain dopaminergic cell bodies which project to nucleus accumbens, the transmitter in the A-10-inferior colliculus projection is not known. It is interesting to note that the substantia nigra, which contains dopaminergic cells projecting to the deeper layers of the superior colliculus (Rinvik et. al., 1976, Brain Res. 112: 388; Hopkins & Niessen, 1976, Neurosci. Letters 2: 253). Thus the existence of a projection from the A-10 area to the inferior colliculus provides support for the idea of a parallelism in the projections of the substantia nigra and the A-10. This projection to the inferior colliculus, an area intimately concerned with auditory function, could play an important role in the affective dissorders in which the A-10 to nucleus accumbens has already been implicated.

(Supported by grants from the MRC of Canada)

652 EFFECTS OF HIPPOCAMPAL X-IRRADIATION ON A DOMINANCE ORDER IN THE RAT. Robert B. Wallace, Gary Freeman*, Jack Werboff*, Robert Graziadei*, and Walter Weiss*. U.of Htfd., Ct. 06117

The technique of focal X-irradiation of the rat hippocampus provides a selective lesion of the granular cells of the dendate gyrus. Anatomically it is possible to limit destruction to hippocampal granule cells since only they are in a proliferative state during radiation with surrounding regions essentially nonproliferative. Behaviorally the advantage of a neonatal lesion is that it allows a developmental approach as to the role of the hippocampus throughout the animal's life.

A number of studies have dealt with possible consequences of hippocampal lesions by assessing behaviors such as two-way avoidance, passive avoidance, and open field behavior. Little work has been done, however, with situations more closely related to behaviors that might normally be required in development. From Altman's hypothesis, we might view the juvenile animal under the protection of its mother as developing exploratory and other skills necessary for independence. Once this state is reached, such uninhibited "play" might well be maladaptive in securing food, protection, and rearing young, thus the development of inhibitory mechanisms would be essential. A study by Kim et al., (1971) supports this notion by showing that hippocampetomized adult rats have little fear in the presence of a live cat and tend to be less aggressive spontaneously or when provoked by shock as evidenced by fear attacks in dyadic pairing. Lorenz (1966) has suggested that aggression may be important in ontogenetic development as a means of self-preservation.

In an effort to pursue some of the issues addressed above, the following dominance study was carried out. Five experimental animals (male Long-Evans hooded rats - random bred in our laboratories) were exposed to focal hippocampal X-irradiation - 150r per day from 2 - 15 post partum; examination of hippocampal anatomy initiated a 70 - 80% reduction in the number of granule cells in the hippocampal dentate gyrus. Five additional males served as non-irradiated sham animals and five served as control animals. Dominance was measured in a plexiglass enclosure with a grid floor across which a 0.5 m.a. scrambled shock was pulsed. Animals were remotely observed and were run in a round robin procedure. Recordings were made of the amount of time each animal spent on the dominance enclosure platform in a trial.

Results indicated that the irradiated animals were submissive to the control animals as reflected in significantly lower total times on platform. Aggressive behavior, even when adaptive in the situation, would appear to be suppressed in the irradiated animals. Some support for the model proposed by Altman was noted.

653 BASAL FOREBRAIN CONTROL OF SWALLOWING. Ananda Weerasuriya*, Detlef Bieger and Charles H. Hockman. Sch. Basic Med. Sci., and Dept. Physiology and Biophysics, Univ. Illinois, Urbana, IL .

In adult cats anesthetized with urethane, electrical stimulation of the basal forebrain facilitated swallowing elicited by electrical stimulation of the superior laryngeal nerve. A stereotaxic mapping study revealed that the facilitatory sites were distributed along the course of the ventral amygdalofugal pathway, specifically its rostral forebrain and hypothalamic components projecting to the anterior amygdalar area, substantia innominata, lateral preoptic area, anterior hypothalamus and nucleus accumbens. The descending pathways mediating facilitatory influences from the nucleus accumbens and amygdala to the brainstem were delineated by placing acute discrete radio-frequency lesions in known target areas of efferents from the two structures. Our results suggest that the amygdala and nucleus accumbens influence swallowing via descending efferents that travel through the lateral hypothalamus.

Reflexly-induced swallowing was also facilitated by microinjections of dopamine (100 µg) at the same amygdalar and accumbens sites which enhanced the reflex response when electrically stimulated. In addition, the reflex response was augmented by close-arterial injections into the forebrain of dopamine agonists L-DOPA (0.2-2.0 mg/kg) and apomorphine (0.04-0.14 mg/kg), an effect that could be blocked by pimozide (0.4 mg/kg, i.v.).

The results from these experiments along with those from an earlier study (Bieger and Hockman: Exp. Neurol., 52: 311-324, 1976) draw attention to the role of the basal forebrain in suprabulbar control of swallowing, and suggest that the observed modulatory influences are mediated by structures known to receive dopamine neuron projections.

Basal forebrain control of swallowing draws attention to the role played by this part of the brain in the integration of olfacto-gustatory information required for the enactment of somato-visceral motor synergies. (Supported by a Grant from the Department of Mental Health and Developmental Disabilities of the State of Illinois)

654 ORIGIN OF THE HIPPOCAMPAL COMMISSURAL PROJECTION IN THE MOUSE USING THE RETROGRADE HORSERADISH PEROXIDASE METHOD. James R. West*, Howard O. Nornes, and Clifford L. Barnes*. Dept. Anat., Sch. Med., University of Iowa, Iowa City, IA 52242, and Sch. Vet. Med. Biomed. Sci., Colorado State University, Fort Collins, CO 80523.

Following small injections of concentrated horseradish peroxidase (HRP) into various regions of the hippocampal formation of C57BL/6J mice, we found retrogradely labeled neurons in regions relatively specific for the injection sites. That is, a mediolateral localization was observed in which labeled cells were found in regions of the regio inferior roughly corresponding to homotopic injection sites in the contralateral hypocampus. Lateral injections labeled cells in CA3a,b regions while more medial injections labeled cells in CA3c-CA4 regions. The most surprising finding was labeled cells in the hilus of the dentate gyrus. Labeled cells in this region were found exclusively following injections that included, or were restricted to, the contralateral dentate gyrus. The HRP procedure also confirms earlier work by several investigators of a moderate spread of the commissural projection along the sepetotemporal axis. Supported in part by IF32 NS05579-01 to JRW and NIH NS 11145-01 and NS 12020-01 to HON.

655 DENTATE GRANULE CELL ACTIVATION BY LATERAL OLFACTORY TRACT STIMLATION. R. Wilson* and O. Steward. Depts of Neurosurgery and Physiology. University of Virginia School of Medicine, Charlottesville, VA 22901.

Historically, the hippocampal formation has been believed to be closely associated with olfactory structures, yet until recently the route from the olfactory bulb to the hippocampal region was unknown. Recent studies, however, have shown that the projections of the lateral olfactory tract (LOT) terminate in layer I of the ventrolateral entorhinal cortex (EC)(Heimer, J. Anat. 103, 1968). Since the EC is the major source of extrinsic afferent input to the hippocampal formation, it has been assumed that this represents the pathway through which olfactory impulses reach the hippocampus. Because the entorhinal projections originate from two cell populations within the EC, one projecting to the dentate gyrus (DG) and the other to regio superior of the hippocampus proper (Steward & Scoville, J. Comp. Neurol. 169, 1976), the question arose whether either or both of these pathways could be activated by olfactory bulb afferents. The hippocampus has a laminated organization making it possible to determine electrophysiologically the synaptic zone activated by a particular afferent. For example, the lateral and medial components of the EC projection to the DG distribute to the outer and middle portions of the granule cell dendrites respectively. These differences are reflected following EC stimulation by a difference in the laminar profile analysis of evoked potential amplitude. In addition, since the laminar profile of evoked potentials in regio superior is easily distinguishable from that in the DG, it is possible to define which of these areas is activated. To investigate the pathways carrying olfactory information, stimulating electrodes were situated in lateral and medial EC and in the LOT. An extracellular recording electrode was advanced in 50 µm steps through hippocampus and the DG, recording the evoked potential following stimulation at each site. Stimulation of the LOT evoked a 14 msec latency potential in the DG. The laminar profile of this potential was virtually indistinguishable from that produced by lateral EC activation but different from the profile evoked by stimulation of medial EC in both dorsal and ventral leaves of the DG. This result suggests the presence of a major polysynaptic pathway from the LOT which travels via the lateral EC to the dentate gyrus. (Supported by USPHS RESEARCH GRANT NO. 1 RO1 NS12333 to O. Steward)

656 EFFECT OF MEDIAN RAPHE STIMULATION ON NEURONAL TRANSMISSION THROUGH THE DENTATE GYRUS IN THE FREELY MOVING AND THE ANESTHETIZED RAT. Jonathan Winson. Rockefeller University, New York, NY 10021.

Previous work in this laboratory in the rat (Winson and Abzug, Science in press) has shown that the efficacy of neuronal transmission through the dentate gyrus following perforant path (pp) stimulation is dependent on behavior. The present investigation was undertaken to ascertain whether serotonergic mechanisms might play a role in producing this effect.

To study the problem, movable electrode devices for stimulating pp and recording from the dentate gyrus were implanted in rats. In addition, a bipolar stimulating electrode was positioned in the median raphe nucleus (MR) to activate the 5-HT pathway originating in MR and innervating the hippocampus. To aid in localization, field potentials in the dentate gyrus elicited by MR stimulation were monitored during implantation. After recovery, animals were tested in the freely moving state. Stimuli were applied to the pp, and evoked action potentials were recorded extracellularly from populations of granule cells during two behaviors, slow wave sleep (SWS) and the still, alert state (Alert). In each behavior, the pp stimulus was either presented alone or was preceded by a stimulus applied to MR, thus resulting in four test conditions at any given value of pp stimulus current, MR stimulus current, and delay time between MR and pp stimuli. Tests were run at a series of pp and MR stimulating currents and at a series of delay times.

As reported previously, granule cell response to pp stimulation alone was greater during SWS than during Alert. Prestimulation applied to MR resulted in a marked augmentation of the already elevated response during SWS, while prestimulation had no effect in the alert state. The augmentation during SWS was dependent on delay time, being prominent at delays of 20 to 65 msec. After tests, animals were anesthetized and retested. Augmentation of the granule cell response following MR prestimulation was also present in the anesthetized condition with a similar dependence on delay time. The results suggest a behaviorally specific serotonergic influence on neuronal transmission through the dentate gyrus.

(Supported by the Harry F. Guggenheim Foundation.)

657 HYPOTHALAMIC AND BRAINSTEM AFFERENTS TO THE HIPPOCAMPAL FORMATION IN THE RAT. J. M. Wyss, Dept. Anat. and Neurobiol., Sch. Med., Washington Univ., St. Louis, MO 63110.

Following moderately large injections of horseradish peroxidase (HRP) into the hippocampal formation (including Ammon's horn, the subiculum and presubiculum) and using benzidine dihydrochloride as the substrate, retrogradely labeled cells have been identified in several regions of the hypothalamus and brainstem, many of which have not previously been recognized as projecting directly to the hippocampal formation. Among the regions showing labeled neurons are: (1) the lateral and posterior hypothalamic areas; (2) the periventricular nucleus; (3) the supramamillary nuclei; (4) the rostral part of the ventral tegmental area; (5) the interpeduncular complex; (6) the superior central nucleus; (7) the dorsal raphe nucleus; (8) the dorsal tegmental nucleus; and (9) the locus coeruleus. In many of these regions the cells were labeled bilaterally, but in nearly every case a significantly greater number of cells was labeled on the side of the injection. The precise site of termination of these hippocampal inputs remains to be determined, but it is evident from these observations that there is a substantial direct input into the hippocampal region from the hypothalamus and rostral brainstem, in addition to the better known indirect input which is relayed in the medial septal/diagonal band complex.

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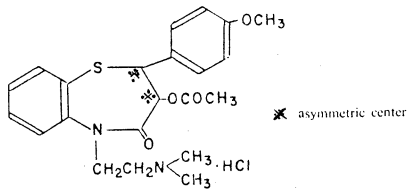
MEMBRANE STRUCTURE AND FUNCTION

658 DIRECT ACTION OF THE CALCIUM BLOCKER DILTIAZEM ON THE CALCIUM CURRENT OF SNAIL NEURON. Norio Akaike*, Kai S. Lee* and Arthur M. Brown. Dept. Physiol. & Biophys., Univ. Tex. Med. Branch, Galveston, TX 77550.

We compared the actions of two drugs, Diltiazem and Verapamil, which block Ca^{2+} currents in excitable membrane. Ca^{2+} currents in single Helix aspersa neurons were examined using our suction pipette method (Nature, 265, 751, 1977) which allows us to combine intracellular perfusion with voltage and current clamp. Ca^{2+} currents are separated by blocking K^+ currents with Cs^+ and Na^+ currents with $Tris^+$ or TTX (10^{-5} g/ml). The action potential in isolated neurons has a threshold but is also voltage-dependent. Diltiazem (10^{-6} to 10^{-4} M) and Verapamil (10^{-5} M) decreased the amplitude of the isolated soma spike markedly, reduced upstroke velocity, prolonged duration and increased threshold potential. Diltiazem and Verapamil blocked Ca^{2+} channels in a voltage dependent manner, their actions being greater at lower voltages. τ_m of Hodgkin-Huxley model was slightly increased while τ_h was greatly increased. The effects of Diltiazem on Ca^{2+} conductance were facilitated in the presence of 10^{-4} M Ni^{2+} and partly reversed by increasing perfusate Ca^{2+} concentrations.

Diltiazem: d-3-acetoxy-cis-2, 3-dihydro-5-[(dimethylamino)-ethyl]-2-(p-methoxyphenyl)-1, 5-benzothiazepin-4(5H)-one hydrochloride.



Diltiazem

659 EFFECT OF LOW-SODIUM SOLUTIONS ON CONDUCTANCE IN THE GIANT ABDOMINAL NEURON (R_2) OF APLYSIA. James P. Apland and David R. Livengood. Department of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Low-sodium external solutions have been reported by various authors to cause an increase, a decrease, or no change in conductance of molluscan neurons (Marmor, Prog. Neurobiol. 5(2):169, 1975). A careful investigation was undertaken to establish whether a conductance change could be demonstrated consistently in the R_2 cell and, if so, to establish the ionic mechanism involved. Numerous sodium substitutes, including Tris, mannitol, magnesium-mannitol, glucosamine, tetraethanolammonium, tetramethylammonium, bis(2-hydroxyethyl)dimethylammonium, choline and arginine were used. Ramp-generated current-voltage plots were used to establish slope conductances. A conductance increase was consistently observed with most of the substitutes. This change could be blocked by extracellular application of 30 mM cobalt chloride. These results imply that calcium is involved in the phenomenon. In some experiments no conductance change was observed in low-sodium solutions. In these experiments a conductance decrease could be demonstrated following application of cobalt. This suggests that a conductance increase, which could be blocked by cobalt, was masked by a decreased sodium conductance brought about by removal of external sodium. It is not yet clear whether the conductance increase in low-sodium solutions is due to a direct increase in calcium conductance or to a calcium-mediated increase in potassium conductance. It was also observed that application of cobalt abolished anomalous rectification in these cells. This suggests that anomalous rectification, which is dependent on external potassium, may involve a calcium-mediated increase in potassium conductance.

660 MECHANICAL ACTIVATION AND ELECTROPHYSIOLOGICAL PROPERTIES OF INTERCOSTAL MUSCLE FIBERS FROM MALIGNANT-HYPERTHERMIA-SUSCEPTIBLE (MHS) PIGS. S. H. Bryant and I. L. Anderson*. Dept. Pharmacol. & Cell Biophys., Univ. Cincinnati Col. Med., Cincinnati, OH 45267, and Col. Vet. Med., Oklahoma State Univ., Stillwater, OK 74074.

The triggering of hyperthermia and the abnormal contraction of muscle from MHS pigs are believed to be due to some defect in the excitation-contraction coupling that leads to elevated calcium levels in the myoplasm. In an effort to quantify the abnormality in the untriggered state we examined the kinetics of mechanical activation of intercostal muscle fibers, controlling the membrane potential with a 2-electrode voltage-clamp and blocking action potentials with tetrodotoxin as described by Adrian et al. (J. Physiol.240:207,1969). External intercostal biopsies were removed from MHS Poland-China and control pigs under anesthesia provided by ketamine, thiopental and nitrous oxide. Small bundles of intact fibers were dissected from the biopsy and studied with microelectrodes in vitro at 38° C. Fibers were depolarized in steps from a holding potential of -90 mV and the membrane potential necessary to produce a microscopically visible threshold contraction was recorded for a series of pulse durations. The membrane potential versus pulse duration produced a "strength-duration" curve for mechanical activation. The mean rheobase for control fibers was -54 mV with the curve leveling off between 100 and 500 msec., whereas the mean curve for MHS fibers was markedly shifted in an approximately parallel manner to a rheobase of only -86 mV close to the holding potential. Dantrolene sodium (2.5 mg/L) added to the bath shifted the strength-duration curve of the MHS fibers toward control values with a rheobase of -54 mV, and the control curve was shifted to a rheobase of -31 mV. The MHS curve in dantrolene was steeper than control fibers in dantrolene at short durations. Cable parameters were also measured in both types of fibers. Membrane resistance was higher and membrane capacitance was lower in MHS fibers compared with control (1218±80 and 582±54 ohm·cm^2; 5.6±0.5 and 10.4±0.9 uF/cm^2, respectively). The strength-duration curves for mechanical activation can be fit reasonably well by a model that assumes that the rate of calcium release from the SR is proportional to the amount of voltage-dependent charge movement in the T-system, the uptake of calcium is first order or saturable, and the threshold calcium concentration is constant and similar in both types of fibers. The lowered mechanical threshold that we found in MHS fibers is best fitted in the simulation by a defect that leads to altered release of calcium. (Supported by USPHS, NIH Grant NS-03178).

661 DOES THE ENDOLYMPHATIC POTASSIUM DEPOLARIZE THE HAIR CELL MEMBRANE? Ruben Budelli* and Humberto Bracho*. (SPON: Douglas Junge). Dept. Head & Neck Surg., Sch. Med., UCLA, Los Angeles, CA 90024.

The hair cells in the vertebrate inner ear, face two different ionic environments. On the innervated side, these cells are in contact with perilymph (a low potassium fluid) and on the ciliated side with endolymph (a high potassium fluid). If the hair cells in the inner ear have membrane potentials dependent on the external potassium concentration, they would be depolarized by the high endolymphatic potassium. To test this possibility we used the macula sacculi in Necturus maculosus to measure the membrane potentials at different potassium concentrations in vitro and in vivo. In the in vitro experiments the saccular wall which contains the macula was excised and placed in a double chamber, in such a way, that the hair cells ciliated side was facing upwards and the solutions on each side of the neuroepithelium could be exchanged independently for solutions with different potassium concentrations. The results show that the neuroepithelial cells in the macula sacculi are depolarized when the potassium concentration is raised on either side of the neuroepithelium. To find out if these cells are depolarized in the normal animal, we recorded the membrane potentials in the neuroepithelial cells in vivo, with the labyrinth intact and after removal of the otolith and replacement of the endolymph by perilymph. The neuroepithelial cells have a larger membrane potentials when the endolymph was substituted by perilymph. An equivalent circuit which explains some of the experimental results is the following:

$Vm = V_1 + Rm.i$

$i = (-V_1 + V_2)/(Rm + R'm + Re)$

if:

$R'm = Rm$ and $Re \quad Rm$:

$Vm = \frac{1}{2}V_1 + \frac{1}{2}V_2$.

Where:

V_1, V_2= Potassium dependent batteries

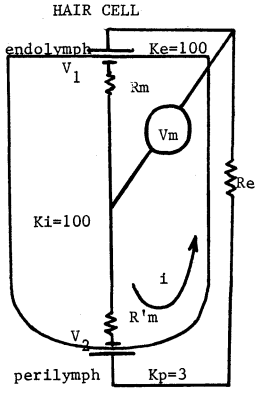
Rm, R'm= Potassium dependent membrane resistance.

Re= Epithelial resistance other than Rm.

Ke= Potassium(endolymphatic)

Kp= Potassium (perilymphatic)

Ki= Potassium (intracellular)



662 FATTY ALDEHYDE AND FATTY ACID COMPOSITION OF NORMAL HUMAN CNS AXOLEMMA-ENRICHED FRACTIONS. V.P. Calabrese, G.H. De Vries and W.J. Zetusky*. Depts. of Neurology and Biochemistry, Med. Coll. of Va. and McGuire VA Hospital, Richmond, VA 23298

Axolemma-enriched fractions from normal human white matter were prepared by osmotically shocking myelinated axons and separating them from myelin on a discontinuous density gradient of 0.8 M, 1.0 M and 1.2 M sucrose. The lipid of the 1.0 M/1.2 M axolemma-enriched fraction was extracted using chloroform-methanol 2:1 v/v and the GPE and GPC fractions were separated by thin layer chromatography. The GPE plasmalogen aldehydes of the 1.0/1.2 fraction and myelin were quantitatively hydrolyzed and isolated by thin layer chromatography using benzene as the solvent. The distribution of plasmalogen aldehydes was determined by GLC of both the free aldehydes and dioxalene derivatives on a DEGS column. Methyl esters of the GPE and GPC fractions were prepared with sodium methoxide, extracted into hexane and analyzed by GLC on a DEGS column.

The myelin aldehydes were comprised of 25% C16:0, 3% C17:0 (?), 18% C18:0 and 54% C18:1. The 1.0/1.2 fraction consisted of 23% C16:0, 5% C17:0(?), 23% C18:0 and 49% C18:1. By contrast CNS "grey matter" GPE aldehydes consisted of 3% C14:0, 1.8% C16:0, 2% C17:0(?), 60% C18:0 and 17% C18:1. For grey matter the weight ratio of C18:1/C18:0 was 0.30 while for myelin it was 3.02 and the 1.0/1.2 fraction had a ratio of 2.14.

Analysis of the GPE and GPC fatty acids showed they were made up primarily of the C16 and C18 family of fatty acids with lesser amounts of what is tentatively indentified as C20:1, C20:2, C20:4 and C24:0. In addition the GPE (1.0/1.2 fraction) contained significant amounts of C22:6 which was virtually absent in the myelin fraction. There were quantitative variations in the distribution of fatty acids from preparation to preparation. In one preparation myelin GPE had a 20:1/20:2 ratio of 2.4 which is similar to what others have found while the analogous lipid in the 1.0/1.2 fraction had a 20:1/20:2 ratio of 0.32.

The results indicate that the axolemma-enriched fraction showed a fatty aldehyde pattern very similar to myelin rather than the cell bodies from which they originate. The difference in the GPE fatty acid profile of the myelin and 1.0/1.2 fraction suggests that there is little contamination of the 1.0/1.2 fraction with myelin lipid. The data suggest that the 1.0/1.2 fraction aldehyde is an intrinsic axolemmal component. The similarity in the GPE aldehyde patterns may indicate a close metabolic relationship between myelin and axolemma.
(Supported by NIH grant NS 10821-04 and a grant from the Multiple Sclerosis Society RG1117-A-1).

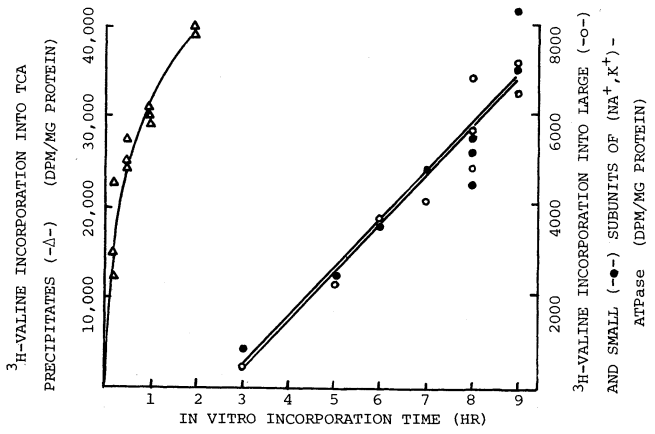
663 SYNAPTIC VESICLES: QUANTITATION OF PURITY AND CHARACTERIZATION. Steven S. Carlson*, John A. Wagner* and Regis B. Kelly (SPON: Walter M. St. John). Dept. Biochem. & Biophys., U. Cal., San Francisco, CA 94143.

Prior to biochemical and biophysical characterization of synaptic vesicles, it is essential to determine their degree of contamination by other membranes. To this end we have developed biophysical techniques for estimating vesicle purity in addition to techniques for vesicle purification. Synaptic vesicles of approximately identical properties have been purified from the electric organs of both Torpedo californica and Narcine brasiliensis, by differential centrifugation of homogenates, followed by flotation on equilibrium sucrose density gradients and molecular seive chromatography on a Controlled Pore Glass column (3,000 A pore size). If necessary, final purification can be achieved on equilibrium glycerol density gradients (vesicle density = 1.10 $g.cm^{-3}$), since glycerol density gradients measure membrane density, unlike sucrose gradients where the density is largely determined by the internal volume of the vesicle (vesicle density = 1.05 $g.cm^{-3}$). At this state the vesicles are over 85% pure, using the criteria of size, charge, and density. The size distribution is best measured by boundary sedimentation in the analytical ultracentrifugation where U.V. absorbing material moves as a single boundary of 100S. Protein, lipid, and acetylcholine coincide on preparative velocity sedimentation in 5-25% glycerol gradients. Charge is measured by electrophoresis in Ficoll density gradients (2-10%). Again, acetylcholine, protein and lipid move as a single component migrating towards the anode at approximately 2.2 x 10^{-3} cm^2/volt-min. Finally, protein, lipid and acetylcholine coincide after equilibrium sedimentation in 20-50% glycerol gradient.

The specific activity of the pure vesicles appears to be between 4 and 6 μmoles acetylcholine/mg protein. Characterization of phospholipid, protein and nucleotide composition of the vesicle has allowed us to estimate a molecular weight of approximately 10^8. From the $S_{20,w}$, and the diameter of the vesicle, the calculated molecular weight is 1.2 x 10^8. Stimulation of the electric organ to exhaustion leads to markedly reduced yield of acetylcholine vesicles, indicating that the purified vesicles are indeed those involved in release. Supported by NIH grant NS 09878. SC (NS01365) and JW (NS05092) are NIH Postdoctoral Fellows.

664 SYNTHESIS OF (Na^+,K^+)-ACTIVATED ADENOSINE TRIPHOSPHATASE. Lynn Churchill and Lowell E. Hokin*, Dept. Pharmacol., Univ. of WI Med. Sch., Madison, WI, 53706.

The synthesis of (Na^+,K^+)-activated adenosine triphosphatase ((Na^+,K^+)-ATPase) has been studied in the electric organ of the electric eel, Electrophorus electricus. In vivo injections of L-(3,4-(n)-^{3}H)-valine into the Main organ and incubations in vitro of this label into isolated groups of Sachs organ cells have been utilized to label the enzyme. Purification of the enzyme has been achieved utilizing modifications of previously published procedures (Dixon and Hokin, Arch. Biochem. Biophys. 163, (1974) 749). In vitro incorporation into the trichloroacetic acid (TCA) precipitate of homogenate (-Δ-) and the large (-o-) and small (-●-) subunits of (Na^+,K^+)-ATPase is illustrated in Fig. 1. The TCA precipitate shows immediate and rapid incorporation; whereas, the (Na^+,K^+)-ATPase subunits show a 2.5-3 hour lag period prior to incorporation. Similar results were observed with the in vivo injections, demonstrating that the lag time is not an artifact of Sachs organ cell isolation. Since our isolation procedure could select for (Na^+,K^+)-ATPase subunits that have already assembled into the plasma membrane enzyme, the lag period could represent the time required for transport and assembly of the subunits. Similarity in the lag period for the large and small subunits suggests that either the routes of transport are similar or assembly into the plasma membrane is the rate-limiting step.



665 MEMBRANE STRUCTURE OF DEVELOPING CHICK MUSCLE FIBERS IN REGIONS WITH HIGH AFFINITY FOR α-BUNGAROTOXIN. S. A. Cohen and D. W. Pumplin. BBB, NICHD, and LNNS, NINCDS, NIH, Bethesda, Md 20014.

A method employing polyvinyl alcohol for the freeze-fracture of monolayers of cells in tissue culture (Pauli et al, JCB 72,763) was modified for use with low-density cultures of chick muscle cells. The method allowed cells to be grown in normal fashion on collagen-coated glass cover slips, and fractured essentially all the muscle fibers along their entire lengths, yielding complementary replicas of nearly 50% of the membrane. Thus, we could observe variations in structural features over large areas of membrane, as well as study those features which occurred relatively rarely. Furthermore, we could reliably localize, in replicas, regions of fibers which were previously observed by high-resolution Nomarski and fluorescence microscopy. Structural features included openings in the membrane, represented by pits (25 nm diameter) in the cytoplasmic leaflet and by corresponding "volcanos" in the external leaflet. These were often found in groups of 2-7 having a nearest-neighbor spacing of about 85 nm. External leaflets had a relatively low density of randomly-dispersed particles in a range of sizes. Cytoplasmic leaflets had two distinctive types of larger particles and a background of randomly-distributed smaller particles. Round-topped particles (7 nm) were found in loose aggregates (1000/μm^2) associated with slightly curved ridges. Angular particles (10 nm) were most conspicuous in closely-packed clusters (2500/μm^2) in which these particles were arranged in rows. In fibers of control cultures, such clusters contained 20-50 particles, and were spaced at random intervals >1μm. Fibers grown in the presence of tetrodotoxin, an agent known to increase the number of cholinergic receptors, contained larger areas of membrane (2-5 μm diameter) in which angular particles were numerous. Such areas contained a number of clusters of 5-50 particles each. They were sharply defined by the edges of the outermost clusters rather than by a gradually decreasing density of individual particles. Membranes appeared relatively smooth, and other types of particles did not aggregate, indicating that the clusters were not an artifact of poor freezing. Furthermore, using the fluorescent label tetramethylrhodamine coupled to α-bungarotoxin, we have identified (in cultures treated with TTX) regions of muscle fibers having a high density of cholinergic receptors. When freeze-fracture replicas of these fibers were examined, groups of clusters of large angular particles were found in the specific locations previously identified by their fluorescence. The association of aggregates of large angular particles with binding of a ligand specific for cholinergic receptors suggests that these particles may be the ultrastructural correlate of these receptors.

666 UDP-GALACTOSE: CERAMIDE GALACTOSYLTRANSFERASE IN A RAT CNS AXOLEMMA-ENRICHED FRACTION. E. Costantino-Ceccarini, A. Cestelli* and G. H. De Vries. Dept. of Neurology, Albert Einstein Coll. Med., Bronx, N.Y. 10461 and Dept. Biochemistry, Med. Coll. Va., Richmond, Va. 23298.

A CNS axolemma-enriched fraction (AXL) was prepared from 25 day old rat brain by density gradient separation of an osmotically shocked preparation of purified myelinated axons. This fraction has been shown to be enriched in surface membrane marker enzymes and shows specific binding of tetrodotoxin (Trans. Am. Soc. Neurochem. 7:223, 1976). Since more than 20% of the lipid in the axolemmal fraction is galactolipid we were interested to know whether some of the galactolipid could be synthesized by the AXL itself. AXL consistently showed activities of galactosyl transferase which were similar to that of microsomes. Since the axolemma is derived from a different cellular type we investigated the possibility that the enzyme could be different from that found in the myelin and microsomes (Brain Res. 93, 358, 1975). No differences in the pH optimum have been found. However some differences were found in the divalent cation requirement for optimal activity. The effect of increasing temperature on the activity of the enzyme present in the different fractions was investigated. The rate of inactivation of the enzyme present in AXL was different from that of either myelin or microsomes. These data suggest that the UDP-galactose: ceramide galactosyltransferase in the axolemma-enriched fraction is different from that of the myelin or microsomes. (Supported by NIH grants NS 10821-04, NS 10885, NS 12807: NSF grant BNS 76 00925, and a grant from the Alfred P. Sloan Foundation.)

667 KINETICS OF LOCAL ANESTHETIC ACTION IN VOLTAGE-CLAMPED FROG MYELINATED NERVE. Kenneth R. Courtney, Joan J. Kendig* and Ellis N. Cohen*. Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305.

Local anesthetic agents block nerve conduction by complexing with a receptor site associated with the sodium channel. Previous studies have shown that lipid insoluble, quaternary local anesthetic analogues can bind to and unbind from the receptor only when the channels are open. This requirement is reflected in the observation that the increment of sodium channel block produced by a channel-opening depolarization (frequency-dependent block) does not decrease over time. While local anesthetics of low lipid solubility such as QX-572 (quaternary) and GEA 968 (tertiary) also preferentially interact with open channels, they show in addition a slow interaction with closed channels. Frequency-dependent increments in channel block do diminish over time, relaxation time constants for these two agents ranging from 10 to 100 sec for doses which block about half the sodium conductance in the rested nerve. Lidocaine, a local anesthetic agent of intermediate lipid solubility, displays a faster interaction with closed channels, relaxation time constants measuring 0.1 to 1 sec at equi-effective doses. These observations suggest that the rapidity of interaction of local anesthetic agents with closed channels may be correlated with lipid solubility. This hypothesis has been tested by exposing voltage clamped frog nodes of Ranvier to bupivacaine, a local anesthetic agent of high lipid solubility at a dose (50μM) which blocked about half the sodium conductance in the rested nerve. However, this agent was found to have a slow rather than a fast interaction with closed channels, not unlike that observed for the low lipid soluble agents QX-572 and GEA 968, and much slower than that observed with lidocaine. The relaxation time constant associated with binding to and unbinding from closed channels was about 50 sec for nodes held at -80 mv and 10°C. We therefore propose that local anesthetics of intermediate lipid solubility show the greatest facility for fast interactions with closed channels, while agents at both the low and high extremes of lipid solubility react relatively slowly. This information should prove useful in determining the microscopic nature of the local anesthetic binding site in the sodium channel. These characteristics are of importance with respect to the frequency-dependent blocking capabilities of local anesthetics in nerve and cardiac tissues, and thus to the analgesic and antiarrhythmic properties of these agents. (Supported by NIH grants GM-22113 and NS-13108)

668 INTRAMEMBRANOUS PARTICLE CHANGES IN TRITON X-100 TREATED PNS MYELIN. M. J. Cullen, R. G. Peterson and H. deF. Webster. NIH, Bethesda, MD 20014 and Dept. Neurobiol. and Anat., Univ. Texas Med. Sch. at Houston, Houston, TX 77025.

Sciatic nerves from young mice were incubated for 4-8 hrs. in 0.5% Triton X-100 in 0.5M ammonium acetate, a solution known to solubilize P_1 and P_2 myelin basic proteins. As previously noted (Peterson, 1976), treated nerves showed extensive splitting and unraveling of the myelin sheath along the major dense line. Some small areas of compact myelin remained. In freeze-fracture replicas, areas of myelin with lamellar splitting were free of intramembranous particles while areas of particle-rich membrane were associated with the patches of compact myelin membrane. Short fixation of 15 mins. to 2 hrs. was sufficient to stabilize the myelin membrane and prevent the Triton X-100 effects even when incubation was extended to 20 hrs. Controls, both normal and 0.5M ammonium acetate treated nerves, had predominantly conpact myelin sheaths with intramembranous particle-rich membrane leaflets.

The data suggest that Triton X-100 alters the compact structure of myelin. In areas where lamellae are split and separated, there is a loss of intramembranous particles. Some of these intramembranous particles may be associated with the P_1 and P_2 proteins believed to be located in the major dense line region of compact myelin.

669 HYPEROXIA AND MEMBRANE POLARIZATION: COMPARED WITH ANOXIA. John Cullerton* and Jacob Zabara. Dept. Physiol./Biophysics, Temple University Hlth. Sci. Cntr., Philadelphia, PA 19140.

Although it has been demonstrated that the compound action potential is depressed in hyperoxia, the relationships to membrane polarization and similar effects in anoxia remain unknown. The compound action potential (A.P.) and the demarcation potential (D.P.) from frog (Grass and Bullfrog) sciatic nerves were amplified and displayed on a Tektronix RM 564 dual channel storage oscilloscope via a Tektronix RM 122 preamplifier. Stimuli and polarizing currents were generated by a dual channel Grass S 8 stimulator with two stimulus isolation units. Continuous recording was achieved by construction of leads in an epoxy seal in the wall of the pressurization tank (Bethlehem Corporation) ending in silver-silver chloride electrodes. Temperature was measured with a mercury bulb thermometer placed in the pressure tank and observed through a glass port. The pressure was in pounds per square inch gauge and controlled by adjusting the pressure-reducing valve at the oxygen cylinder. In order to develop reliably a stable D.P., isotonic KCl was applied to the tied end of the nerve while it was stored overnight with the rest of the nerve kept on Ringer's soaked gauze.

The effect of hyperoxia (9 ATA) to diminish the A.P. and D.P. could be reversed by continuous anodal polarization, which, over a period of minutes, restored the potentials to their control value. Discontinuance of the anodal polarization resulted in a resumption of the decline of these potentials. This effect of anodal polarization could be exhibited for a period of several hours. The threshold varied inversely with the change in the D.P. A steady and sizeable increase in threshold indicates the initiation of membrane deterioration. Polarizability, as tested by brief rectangular pulses producing catelectrotonus, decreases when the hyperoxic effect is unopposed by anodal polarization and corresponds directly to the change in the D.P. This anodal polarization can antagonize the hyperoxic caused decay of the A.P., but not restore it after conduction has ceased. The decrease in the D.P. indicates membrane depolarization as is observed in anoxia. Further, the action of anodal polarization to maintain membrane polarizability and the A.P. is similar in anoxia.

If reducing equivalent depletion is the cause of the depolarization, the anodal polarization may act to supply (unstably) reducing equivalents. Also, metabolic depletion appears to produce a faster time course of oxygen toxicity in this preparation. Depolarization may expose the membrane to an accelerated oxygen toxic effect. Thus, destructive damage of the plasmalemma is not primary in either hyperoxia or anoxia, but rather appears to arise from a deficiency in the metabolic supply to the membrane.

670 SCORPION NEUROTOXINS AS AFFINITY REAGENTS FOR SOLUBILIZED VOLTAGE DEPENDENT SODIUM CHANNEL ELEMENTS. W. J. Culp*, D. Welch*, D. Mayka* and J. Taylor*. (SPON: H. Borison). Dept. Biochem., Dartmouth Med. Sch.

The physiological actions of neurotoxins purified from the venoms of two species of scorpion have been defined by intracellular recording from a variety of excitable tissues and by voltage clamp studies on the frog Ranvier node. Purified Leiurus quinquestriatus neurotoxins selectively inhibit the onset of sodium inactivation. This inhibition of "h" gating is reversible. Centruroides sculpturatus neurotoxins specifically alter the voltage dependence of sodium activation, or "m" gating, as described for the whole venom of this species by Cahalan (J. Physiol. 244, 511 (1975)). Biochemical studies indicate that these physiologically distinct neurotoxins have a number of structural similarities including molecular weight, isoelectric pH values, spectral properties and sensitivity to oxidation.

Defined neurotoxins from both species of scorpion have been successfully radiolabeled. These toxins have been employed as affinity reagents in an attempt to identify "m" and "h" gating components solubilized from eel electroplaque membrane. Sucrose gradient studies demonstrate the presence of presumptive voltage dependent sodium channel gating elements in these extracts which exhibit high affinity neurotoxin binding. Related studies have provided evidence for a somewhat lower affinity interaction between Leiurus neurotoxins specific for "h" gating and the acetylcholine receptor, both in situ in clonal muscle cell lines and in detergent extracts of electroplaque membrane.

671 cAMP-DEPENDENT PHOSPHORYLATION OF ENDOGENOUS SUBSTRATES IN SYNAPTIC PLASMA MEMBRANES. A. de Blas, Y-J. Wang and H.R. Mahler. Indiana Univ., Bloomington, IN 47401.

cAMP (0.010 mM) stimulates the phosphorylation of six endogenous protein substrates in synaptic plasma membrans (SPM), using 0.01 mM (^{32}P)ATP as donor. These polypeptides have tentative Mol.wt. of 340,000, 80,000, 75,000, 66,000, 54,000 and 50,000 and are designated proteins (or bands) 1, 2, 3, 4, 5 and 6, respectively. We have followed the presence of these endogenous substrates in different cortex subfractions and find Band 6 to be ubiquitous, while band 4 is restricted to SPM. Analogous phosphorylation of membrane proteins is not seen in purified mitochondria from cortex or in crude mitochondrial fractions from cerebellum or liver. In SPM a peak of phosphorylation is reached for all substrates within 5-10 s. Dephosphorylation of Band 6 is faster (within 30-60 s) than the rest, which remain fully phosphorylated for at least 2 min. When the concentration of ATP is raised to 0.5 mM the phosphorylation of Band 6 decreases sharply with no effect on the other bands. Deoxycholate in concentrations $\geq$ 0.5% inhibits both phosphorylation and dephosphorylation of the cAMP-responsive bands over the cAMP-independent polypeptides. Phosphorylation of Bands 2 and 3 appear most susceptible to this inhibition. Triton X-100 at concentrations $\geq$ 0.5% stimulates phosphorylation of all protein substrates in SPM, except for that in Bands 2 and 3, which appears specifically inhibited. Treatment with this detergent does not lead to the selective solubilization of any of the phosphate acceptors. Phosphorylation stimulated by cAMP of proteins in the SPM is not modified by extraction of the latter with either chloroform-methanol or treatment with phospholipases A and C. However, preincubation of the membrane with either of these enzymes results in stimulation of the phosphorylation of all bands susceptible to this modification, either in the absence or presence of cAMP. All these phosphoproteins appear to be degraded by trypsin. The stimulation of phosphorylation of Bands 1 - 6 exhibits a pH optimum around 6, with bands 5 and 6 the most sensitive to variations in pH. Potassium or phosphate ions stimulate phosphorylation in a non-selective fashion, except for that of Band 6, which is inhibited by phosphate. Different transmitter-related agonists and antagonists for muscarinic and nicotinic cholinergic, and for beta-adrenergic receptors do not affect phosphorylation in the presence, or absence, of cAMP. It is also unaffected by Na and/or K ions.

672 LIPID COMPOSITION OF NORMAL HUMAN CNS AXOLEMMA-ENRICHED FRACTIONS. G.H. De Vries, C.J. Zmachinski*, W.J. Zetusky*, and V.P. Calabrese Depts. of Biochemistry and Neurology, Med. Coll. of Va. and McGuire VA Hospital, Richmond, VA 23298

Axolemma-enriched fractions were isolated from human white matter via a purified preparation of myelinated axons which were osmotically shocked and fractionated on a discontinuous density gradient to yield myelin, and two membrane fractions at the 0.8 M/1.0 M and 1.0 M/1.2 M sucrose interfaces (axolemma-enriched fractions). The myelin lipid is comprised of 25% cholesterol, 45% phospholipid and 30% galactolipid. The 0.8/1.0 axolemma-enriched fraction lipid is comprised to 30% cholesterol, 28% galactolipid plus 42% phospholipid. The 1.0/1.2 axolemma-enriched fraction lipid is comprised of 23% cholesterol, 30% galactolipid and 47% phospholipid. The myelin ethanolamine phosphatides comprised 17% of the total lipid weight with 75% of the total ethanolamine phosphatides in the plasmalogen form; choline phosphatides comprised 11% of the total myelin lipid with 7% of the total choline phosphatides in the plasmalogen form. Sphingomyelin comprised 7% of the myelin lipid with lesser amounts of phosphatidyl serine and phosphatidyl inositol. The 0.8/1.0 fraction has about the same amount of choline phosphatides, phosphatidyl serine and phosphatidyl inositol as myelin but the fraction contains only 14% ethanolamine phosphatides. The 1.0/1.2 fraction is distinctly different in its phospholipid composition from myelin and the other axolemma-enriched fraction. Choline phosphatides comprise 16% of the total lipid with about 15% in the plasmalogen form; ethanolamine phosphatides comprise about 16% of the total lipid with over 50% of the total ethanolamine phosphatides in the plasmalogen form. Sphingomyelin comprises 4% of the total 1.0/1.2 lipid and the levels of phosphatidyl inositol and phosphatidyl serine are similar to myelin and the 0.8/1.0 fraction. Expressed on a weight basis the ratio of choline to ethanolamine phosphatides is 0.64 in myelin, 0.83 in the 0.8/1.0 fraction and 1.06 in the 1.0/1.2 fraction. Gangliosides comprise 0.5% of the total lipid in the 0.8/1.0 fraction and 1% of the total lipid in the 1.0/1.2 fraction. We conclude that the lipid composition of the axolemma-enriched fractions resembles myelin in the galactolipid and plasmalogen content, however, the composition is distinctly different in having a higher lecithin and ganglioside content and somewhat lower ethanolamine phosphatide content. The lipid composition and the low levels of both 2'3' cyclic nucleotide 3' phosphohydrolase and myelin basic protein lead us to believe that not all the galactolipid in these fractions is due to myelin contamination but that it is an intrinsic component of the mammalian axolemma.

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673 REGIONAL DISTRIBUTION OF IONIC MECHANISMS IN THE BARNACLE PHOTORECEPTOR MEMBRANE. Duane R. Edgington* and Ann E. Stuart. Dept. Neurobiology, Harvard Med. School, Boston, MA 02115

Properties of the somatic, axonal and presynaptic membranes of photoreceptors of the giant barnacle (B. nublius) were compared by recording intracellularly from each region. Each of these regions could be superfused separately or surgically isolated from one another. Previous work has shown that these cm-long neurons have a high resistivity membrane that allows voltage changes generated by light in the somata to spread decrementally along the axons to the presynaptic terminals. In the terminals, a voltage-sensitive Ca current apparently associated with transmitter release is normally opposed by a tetraethylammonium (TEA)-sensitive K current but can become regenerative in mM TEA (Ross and Stuart, Biol. Bull. 151, 427 (Abs) 1976 and in prep.). We show here that the Ca current and the TEA-sensitive K current, and in addition, a TEA-insensitive K current, are present in the soma and axon as well as in the terminals.

Although the axons conduct decrementally in normal saline, a Ca action potential (AP) can be elicited in the axon in the presence of TEA. The axonal AP has a slower rate of rise and higher threshold than the terminal AP. The axonal AP is blocked by 5 mM Co while 15-20 mM Co is required to block the terminal AP; the dose-response relations suggest that Co is competing with Ca in both regions. Thus a given depolarization appears to generate a smaller Ca current in the axon than in the terminals. The soma also displays a Ca AP when bathed in TEA, with properties similar to the axonal AP. We conclude (1) that Ca channels are not localized exclusively in the synaptic membrane but (2) that they are present in lower density in the soma and axon than in the terminals.

The effects of TEA on Ca APs indicate that TEA-sensitive K channels must also be present in all regions of the membrane. In the terminal, the AP is followed by an undershoot of long duration (seconds) that is present even in 400 mM TEA. This undershoot is due to a conductance increase whose reversal potential varies with extracellular K concentration. Ca APs elicited in somata and axons with TEA are also followed by long-lasting undershoots. Thus the TEA-insensitive K channels like the TEA-sensitive channels and Ca channels are present throughout the photoreceptor membrane.

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674 SODIUM-POTASSIUM ATPASE IN BRAIN CAPILLARIES. Howard M. Eisenberg and Robert L. Suddith*. The Division of Neurosurgery and The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

In these studies we examined the capacity of brain capillaries to actively transport Na^+ and K^+. In other tissues it has been clearly shown that active transport of these ions is intimately related to Na-K ATPase and in fact that the magnitude of this transport may be related only to the amount of the enzyme that is present. Brain capillaries were isolated from mouse cerebral hemisphere by a process of homogenization, serial filtration and differential centrifugation. Subcellular fractions were incubated with ATP in the presence or absence of ouabain (1mM). The activity of Na-K ATPase, expressed as uM of inorganic phosphorous released per mg. of protein per hour was taken to be the difference in activity found in the absence and in the presence of ouabain. Na-K ATPase activity was similarly determined in subcellular fractions made from human umbilical endothelial cells and from C_6 astrocytoma cells. Na-K ATPase activity in the brain capillary preparation was 9.07 ± 1.98. This activity was considerably greater than that measured in preparations made from umbilical vein endothelium, 0.024 and slightly greater than that determined in preparations of C_6 astrocytoma cells, 1.16. The amount of phosphorous liberated by the brain capillary preparation increased with time and increased with substrate concentration following Michaelis-Menton kinetics. Radiographs made following incubation of unlysed brain capillaries with tritiated ouabain showed ouabain binding along the capillary segments.

There are reasons to suggest that similar active processes are involved in the formation and initial regulation of K^+ concentration in cerebrospinal fluid (CSF) and brain extracellular fluid (ECF). The fluids are similar, if not identical. Brain ECF has been shown to be a significant source of CSF, a flow demonstrated even when osmotic conditions favored a flow in the opposite direction. An active ouabain sensitive Na^+ transport is the driving force for CSF formation by the choroid plexus ependyma and that although regulation of CSF K^+ concentration is complex, there is evidence for regulation of the nascent fluid at the choroid plexus. Although brain ECF and CSF are similar, it is unlikely that the CSF has a total influence on K^+ concentration of the brain ECF. The finding of significant levels of Na-K ATPase in brain capillaries supports this idea of similar active processes and suggests that brain capillary endothelium may be in some ways functionally similar to choroid plexus ependyma.

Supported by PHS Grants NS07377 and NS11255

675 STUDIES ON GANGLIOSIDE PATTERNS AND CHOLERA TOXIN-PEROXIDASE LABELING OF AGGREGATING CELLS FROM THE CHICK OPTIC TECTUM. Edgar L. Engel, John G. Wood, and Frances I. Byrd*. Dept. Anat., Univ. of Tenn. Ctr. for the Hlth. Sci., Memphis, TN. 38163.

The changes in ganglioside patterns in aggregating cells from the chick optic tectum have been examined, and these changes correlated with the cytochemical localization of cholera toxin-peroxidase binding sites on these cells and in the developing rat cerebellum. Tectal cells from 6- and 8-day embryos were studied in reaggregating cultures (Seeds, Proc. Natl. Acad. Sci., USA. 68: 1858, 1971), since changes in tectal cell surface properties have been shown to occur during these embryonic stages (Gottlieb, et al., Proc. Natl. Acad. Sci., USA. 71: 1800, 1974). Thin layer chromatograms (TLC) of gangliosides from cultures 1, 7 and 14 days in vitro (DIV) were compared with patterns from optic tecta of corresponding embryonic ages. Aggregates and dissociated cells were also fixed and treated with cholera toxin-peroxidase to localize its presumed binding site, G_{M1} (Manuelidis, et al., Science 193: 588, 1976). These in vitro cytochemical studies were compared to labeling of fixed tissue slices from developing rat cerebellum.

Cultures from both 6- and 8-day tectal cells showed increases in the amount of ganglioside-sialic acid, but these increases lagged behind those occurring in vivo. Densitometric scans of TLC patterns of gangliosides from dissociated cells, aggregates and optic tecta revealed changes in the relative proportions of the various gangliosides as development proceeds both in vitro and in vivo. Early stages in the formation of aggregates have G_{D3} as the predominant ganglioside. After 1 week in culture, G_{D3} has decreased proportionately, and G_{D1a} has become the major ganglioside. G_{M1} and G_{T1} increase from initially low levels over the time periods studied. Parallel changes in ganglioside profiles occur in vivo.

Cholera toxin-peroxidase bound uniformly to the processes and soma of aggregating, 8-day tectal cells throughout the culture times studied. Labeling of dissociated cells and aggregates (1 DIV) from 6-day tecta appeared diminished in comparison to the older stages. In vivo studies on the developing rat cerebellum demonstrated labeling of plasma membranes of all cell types. This localization includes plasma membranes of developing processes, growth cones, synaptic membranes and the synaptic cleft.

The cytochemical observations indicate a rather uniform distribution of cholera toxin-peroxidase receptors on developing neuronal membranes. Other changes in gangliosides observed may be related to such events as the formation of synaptic junctions in tectal cell aggregates and the optic tectum. Supported by USPHS Grants 5T01-GM00202 and NS-12590; Sloan Fellowship (JGW).

676 IMPLICATIONS OF A CURRENT-CONDUCTANCE CORRELATION ON THE OPERATION OF THE K-CHANNEL OF THE SQUID GIANT AXON Jurgen F. Fohlmeister* (SPON: R.E. Poppele). Lab. of Neurophysiology, Department of Physiology, University of Minnesota, Minneapolis, MN 55455 and Marine Biol. Lab., Woods Hole, Mass. 02543.

Voltage clamp studies were performed on internally perfused giant axons of the squid Loligo pealei, bathed in artificial sea water solutions containing sufficient TTX to completely block the sodium channel. Potassium current was measured as a function of time with the internal and external K^+ concentrations varied isotonically in the ratios c_K^i/c_K^o = 150/50, 300/50, 300/10 and 450/5 (milliequivalents top and bottom) providing a wide range of E_K. At selected times following the test clamp step and covering the range 0.5 to 30.0 ms, membrane potential was stepped an additional ΔE = 10mV, and instantaneous conductance $g_K \equiv \Delta I/\Delta E$ was derived. In this way we obtained families of curves g(t), I(t) for test potentials E_T = -20, +5, +30, +55mV (inside relative to outside). Plotting g_K versus I_K for each given E_T, we found that all early time (t<4ms) points fell on the same straight line, independent of the c_K^i/c_K^o ratio. This result is in contrast to an I_K dependence of $g_K(E-E_K)$ with g_K explicitly dependent only on E and t. Long time (t>6ms) points fell on curvilinear lines consistent with periaxonal K^+ accumulation.

According to transport theory g_K^{-1} is proportional to $\int_{\text{ai}}^{\text{o}} \frac{dx}{c_K(x)\,u_K(x)}$ when the membrane is near thermodynamic steady state, $u_K \equiv K^+$ mobility. Under constant field and constant u_K assumptions, the "independence principle" equation of Hodgkin-Katz does predict nearly the correct ΔI's and the accompanying required changes in $c_K(x)$. These assumptions however lead to incorrect slopes of early time g_K vs I_K curves. Modifying the constant field to include an "image force" induced energy barrier (heights in the range 0 to 10 kT) results in no improvement of the g_K vs I_K slopes. A sizeable barrier may in fact lead to unrealistically large diffusion constants or intramembrane concentrations needed to support the required current I_K. The correct g_K-I_K slope results from a non-uniform mobility, $u_K(x)$, such that the mobility is reduced near the axoplasmic side, relative to the periaxonal side.

677 CONDUCTION VELOCITY AFTEREFFECTS OF SPIKE ACTIVITY: QUANTITATIVE STUDIES. Stephen A. George and Peter T. Silberstein*. Amherst College, Amherst MA 01002.

The aftereffects of a given impulse can cause a subsequent one to travel at a different velocity, resulting in changes in interval between the spikes during propagation. The theoretical work described here was designed to provide insight into the mechanisms responsible for the conduction velocity (CV) changes, and to study the effects of the resulting changes in interspike interval on the statistical description of spike trains.

The Hodgkin-Huxley equations for propagated action potentials in the squid axon, and the similar set of equations developed by Dodge and Hille for frog sciatic nerve fibers, were solved on a computer using ordinary iterative methods without approximations. Results were as follows: (i) Conduction velocity of second spike. The squid axon equations predicted substantial refractory slowing of a 2nd impulse initiated within 15 msec after the 1st one, and slightly supernormal CV for a 2nd spike initiated between 15.5 and 24 msec after the 1st one. The maximal effect was a 1.5% CV increase at 19 msec, which is much less than the effect observed in many types of fiber. The effect was even smaller in computations using the Adelman-Palti model for K^+ accumulation around the fiber. The equations for sciatic nerve predicted refractory slowing but no supernormal CV, although that phenomenon has been found experimentally in sciatic nerve fibers. (ii) Excitability and conduction velocity. The magnitude and time course of post-spike CV changes closely paralleled the changes in excitability as determined by threshold computations. (iii) Displacement of initial voltage. In squid, the CV of a single spike decreases monotonically with increasing levels of maintained depolarization of the membrane before the stimulus pulse. In contrast, depolarizations of up to 9 mV caused an increase in the CV of sciatic nerve spikes; for larger depolarizations, the effect was reversed. In summary, the increases in CV during the supernormal period observed in many fibers cannot be accounted for by the membrane mechanisms incorporated in the Hodgkin-Huxley model for impulse propagation, as elaborated for the frog and squid axons.

In a separate study, CV aftereffect data was applied to two types of simulated spike train with known initial statistical characteristics (Poisson trains, and trains generated by a clock with added random delays). The interval histograms and serial correlation coefficients for both types of train showed substantial changes after propagation, in cases with certain initial interval distributions. The changes in spike train parameters were large enough to affect the transmission of information in fibers that use an interval code.

678 RECONSTITUTION OF ACTIVE ION TRANSPORT BY PURIFIED SODIUM AND POTASSIUM ION-ACTIVATED ADENOSINE TRIPHOSPHATASE. Stanley M. Goldin* (SPON: E. Henneman). Harvard Medical School, Boston, MA 02115.

Purified sodium and potassium ion-activated adenosine triphosphatase (NaK ATPase) was reconstituted into phospholipid vesicles. The resulting in vitro transport system consisted of NaK ATPase associated with single bilayer vesicles, primarily 400A-600A in diameter, as determined by Sepharose gel-filtration. An average of only 5% of the total NaK ATPase activity in this system was unreconstituted. About half of the reconstituted NaK ATPase was oriented "inside out" with respect to its in vivo orientation: externally added ATP generated active uptake of Na^+ ions into the vesicles, inhibitable by internally incorporated but not externally added ouabain. The vesicle Cl^- permeability was more than tenfold higher than the Na^+ and K^+ permeabilities; the Na^+ and K^+ permeabilities were equal. Scans and amino acid analysis of SDS gels of the reconstituted NaK ATPase preparation showed that the two polypeptides of the NaK ATPase preparation showed that the two polypeptides of the NaK ATPase constituted ~95% of the vesicle protein; no other protein component was more than 2% of the vesicle protein.

This in vitro transport system was sufficiently characterized to prove that the reconstituted NaK ATPase catalyzes active countercurrent K^+ transport coupled to active Na^+ transport; the stoichiometry of Na^+/K^+ pumped/ATP hydrolyzed is ~3/2/1, as observed in red cell and nerve. The purity and high reconstitution efficiency of the NaK ATPase enable one to unambiguously demonstrate that no protein of molecular weight >12,000 besides the NaK ATPase can be involved in this transport.

679 MORPHOLOGIC INTERRELATIONSHIPS BETWEEN MITOCHONDRIA AND THE ENDOPLASMIC RETICULUM OF NEURONS. Maryanna Henkart. Behavioral Biology Branch, NICHD, NIH, Bethesda, Maryland 20014.

Mitochondria have been thought to be the primary cellular organelle responsible for Ca sequestration in neurons, while results from this and other laboratories have implicated the endoplasmic reticulum (ER) in that function. Thus, it was of interest to study the morphologic interrelationships of these two organelles. Using a stain selective for membranes, the mitochondria and ER have been studied in serial thin sections of mouse spinal cord and dorsal root ganglion cells in culture, in ganglion cells of Aplysia and snail, and in the squid giant axon. Mitochondria are often enwrapped by ER, and there are often images of apparent continuities between the ER and mitochondrial outer membranes. Although these images in thin sectioned material are frequent and suggestive, they are ambiguous because the continuities between the lumens of ER and outer mitochondrial space are smaller than the thickness of a section. In addition to the images of apparent continuities the following observations suggest that the continuities are real: a) Mitochondrial outer membranes sometimes appear to be continuous with the nuclear envelope. b) Mitochondrial outer membranes sometimes form subsurface cistern-like junctions with the surface membrane adjacent to or apparently continuous with subsurface cisterns of ER. c) In swollen or lysed cells membrane continuities can easily be traced between the mitochondrial outer membrane and rough ER. d) In neurons prepared for histochemical demonstration of peroxidase, reaction of product is sometimes found in the space between the inner and outer mitochondrial membranes. This is due to endogenous oxidases associated with mitochondria. In the vicinity of these stained mitochondria, reaction product is sometimes also seen in the nearby ER lumen. Thus, either the endogenous oxidase enzymes are localized both in mitchondria and that small area of adjacent ER, or there is a pathway for diffusion of reaction product from the mitochondria into nearby ER. Freeze fracture images are being studied to clarify the nature of these continuities. These observations demonstrate a morphologic pathway for the direct exchange of materials between the lumen of the ER and the space between the inner and outer mitochondrial membranes. Such continuities may be transient and may not involve all mitochondria.

This interaction between the ER and mitochondria could be important in the control of intracellular Ca, but could be involved in many other cellular processes as well.

680 CALCIUM ACTIVATED AFTERHYPERPOLARIZATION IN HIPPOCAMPAL SLICES MAINTAINED IN VITRO. John R. Hotson*, Philip A. Schwartzkroin, and David A. Prince (SPON: T.A. Pedley). Dept. Neurol., Stanford Univ. Sch. Med., Stanford CA 94305.

In CA1 cells of guinea pig hippocampal slices a 125msec intracellular depolarization pulse elicits repetitive action potentials followed by a prolonged afterhyperpolarization (AHP). The AHP has an amplitude of 2-8 millivolts and a duration of 250-1000 msec. A 15-25% membrane conductance increase occurs during the peak amplitude of the AHP. This conductance change then decreases in parallel with the return of the AHP toward the resting membrane potential.

In contrast, direct stimulation of CA3 recurrent Schaffer collaterals evokes a short duration hyperpolarizing potential in CA1 cells which has previously been identified in vivo as an inhibitory postsynaptic potential (IPSP) (Kandel et al., J NEUROPHYSIOL 24:225, 1961). The hyperpolarization associated with the IPSP, but not the AHP, can be attenuated by intracellular injection of chloride. Combining intracellular chloride injection with extracellular ammonium acetate (2mM) perfusion produces further IPSP attenuation. Ammonium acetate is presumed to cause intracellular chloride accumulation by blocking its active extrusion (Lux, SCIENCE 173:555, 1971). This dissociation of the IPSP from the AHP indicates the latter is independent of chloride and, by exclusion, appears to reflect an increased membrane conductance to potassium ions.

Manganese (2mM), a calcium antagonist, reversibly inhibits the AHP and its associated conductance increase. The preceding depolarization-induced repetitive action potentials are unchanged. Synaptic field potentials and postsynaptic potentials are also blocked by manganese. These data indicate that the prolonged AHP is the result of an increase in membrane conductance to potassium which is activated by a preceding calcium influx. Similar hyperpolarizing potentials have been found in various invertebrate and vertebrate preparations (Barret & Barrett, J PHYSIOL 255:737, 1976). This report is the first description of such an AHP in mammalian cortex. It may have functional significance in limiting high frequency neuronal firing and burst generation in epileptogenesis. (Supported by NIH grants NS06477 and NS12151 to D.A.P. and NS07012 to J.R.H.)

681 GLYCOSYLTRANSFERASE ACTIVITIES IN NORMAL AND DENERVATED MUSCLE PLASMA MEMBRANES. Peter L. Jeffrey* and Stanley H. Appel. Jerry Lewis Muscular Dystrophy Center and the Dept. of Neurology, Baylor College of Medicine, Houston, Tx. 77030

Surface carbohydrates have been implicated in cell-cell interaction and cellular adhesion. Following denervation the muscle cell reacquires its capacity for muscle-muscle as well as nerve-muscle interactions. If carbohydrates play a role in the process of intercellular recognition and adhesion, the surface membranes of denervated muscle would be expected to undergo significant changes with respect to carbohydrate content and glycosyl transferase activity.

Plasma membranes were isolated from extensor digitorum longus, soleus, and gastrocnemius skeletal muscles of normal rats and rats denervated for 7 days. Sialic acid content of sarcolemmal membranes increased from 16 to 40 nanomoles per mg after denervation. Hexose content was found to increase from 280 to 980 nanomoles per mg while total hexosamine increased from 30 to 46 nanomoles per mg.

Sialyltransferase activity was measured by the transfer of radioactively labeled sialic acid from CMP-sialic acid to endogenous acceptor present in the membranes or an exogenous acceptor asialofetuin. Galactosyl transferase was measured by transfer of radioactively labeled galactose from UDP-galactose to endogenous or an exogenous acceptor, asialo-agalacto-fetuin. Both enzymes showed maximal activity at pH 6.8, the galactosyl transferase showed a broader optimal activity from 6.3 to 7.4. Enzymes were linear with respect to both time and enzyme concentration under the conditions employed. Endogenous activities of the transferases were less than 10% of those in the presence of exogenously added acceptors in normal muscle membranes and changed minimally on denervation. Sialyl transferase and galactosyl transferase activities towards the fetuin derivatives were increased two-fold after 7 days' denervation in sarcolemmal membranes.

These increases in carbohydrate content of membrane macromolecules are not paralleled by similar increases in membrane polypeptides or lipids. Since in most membrane systems carbohydrates are asymmetrically located and appear to project to the extracellular environment, it appears reasonable to suggest that the increased carbohydrate content of denervated plasma membranes may participate in the process of cell-cell interaction.

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682 IN VIVO INHIBITION OF SKELETAL MUSCLE SODIUM PUMP BY OUABAIN IN RATS. George Karpati, Andrew A. Eisen* Stirling Carpenter* and Hanna Pappius* Montreal Neurological Institute, Montreal, Canada.

Ouabain administered to Sprague Dawley rats (15mg/kg body weight) intraperitoneally produced marked weakness of most skeletal muscles, while respiration remained adequate. The paresis started at 10 minutes, reached the maximum at 30 minutes and disappeared by 90 minutes. During maximal weakness, femoral arterial blood pressure was normal, and the ECG showed only S-T segment depression. At this time, ouabain concentration in sera was 5.7×10^{-5} M(N=5) and in plantaris muscles 3.2×10^{-6} M(N=12). Sera removed at the height of the paresis showed a marked rise of K^+ (10.85 mM, N=7) and a modest increase of Ca^{++} (11.31 mg%, N=8) while Na^+concentration declined (137 mM, N=7).

In vivo isometric twitch tension was initially (between 5-15 minutes) potentiated to 137% of the control values; then it progressively declined to 0 at 30 minutes and subsequently recovered exponentially to normal values by about 100 minutes post-injection. The amplitude of the compound action potential followed a similar time course of decline and recovery, but there was no initial potentiation. The action potential amplitude and conduction velocity in the mixed ventral caudal nerve remained normal throughout. The muscle fibres showed no abnormality by histochemistry and EM at the height of paresis.

It is concluded that in rats, ouabain induces paralysis by specific inhibition of the sarcolemmal Na^+ K^+ ATPase,in doses which do not appear to significantly impair cardiac rythm or contractility. Lower concentration of ouabain produces positive inotropic effect of skeletal muscle. These effects of ouabain can be utilized as an in vivo assessment of available functional ouabain binding sites in rat skeletal muscles.

683 MOLECULAR ARCHITECTURE OF CNS SYNAPSES: CHARACTERIZATION AND POSSIBLE FUNCTIONS OF CONSTITUENT PROTEINS. Paul T. Kelly and Carl W. Cotman. Dept. Psychobiol., Univ. Calif., Irvine, CA 92717.

The major polypeptides in isolated synaptic junctions(SJ) and postsynaptic densities (PSD) have been analyzed by two-dimensional polyacrylamide gels. Peptide maps of these major synaptic proteins have been compared to muscle actin, cytoplasmic tubulin and the major neurofilament protein. These results show: (1) α- and β-tubulin present in SJ and PSD fractions are indistinguishable to the subunits of cytoplasmic tubulin, (2) the actin in these subsynaptic fractions is the β- and γ-actin isoproteins found in non-muscle cells and α-actin, characteristic of skeletal muscle, is not detectable, (3) the major PSD polypeptide is distinct from all other known fibrous proteins. Because of its biochemical distinctness we have named the major PSD protein "synapsin". It is proposed that synapsin may be the basic structural backbone of the PSD and may serve as the molecular interface between the dendrite's cytoskeletal network and postsynaptic membrane. Intermolecular disulfide bonds appear to play a critical role in this union.

The presence of actin and tubulin suggests that the synaptic junction may have heretofore undiscovered chemo-mechanical properties. However, in order for this to be the case it is necessary to have the enzymes to catalyze such structural changes. It appears that isolated SJ fractions contain a significant amount of Ca^{++}-stimulated and ouabain insensitive ATPase activity. Although the functional role of this ATPase activity is yet unclear it may represent a myosin-like ATPase system which in conjunction with actin and other constitutents could comprise a synaptic mechano-contractile system. Thus, besides serving a static structural role it is likely that the synapse's cytoskeltal network of fibrous proteins can regulate membrane shape and spine configuration at asymmetric synapses. Ca^{++}-stimulated ATPase could also regulate the amount of tubulin associated with synaptic junctional membranes as it appears a similar enzyme does in regulating tubulin polymerization at the mitotic spindle. The activation of the ATPase may originate from changes in intracellular Ca^{++} caused by presynaptic activity. In this way presynaptic activity may alter postsynaptic structure. (Supported by NIH grant NS 08597 and post-doctoral fellowship 1F32 NS 05746.)

684 PHOSPHOLIPID LABELING IN THE NOCTUID MOTH EAR: A MODEL FOR BIOCHEMICAL STUDIES OF TRANSDUCTION. Patricia L. Kilian* and Jochen Schacht, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

The mechanism of transduction of vibratory energy into nerve impulses is the basic question in a molecular theory of hearing. In the mammalian inner ear, sound stimulation induces the receptor potential and numerous other bioelectric phenomena in the organ of Corti: afferent and efferent synaptic transmission, generator and action potentials. Biochemical processes possibly associated with these secondary events would obscure those underlying transduction. In contrast, hearing organs in insects are functionally simple and easily accessible. The tympanic organ of the Noctuid moth seems especially suited for such investigations. There are two independent auditory receptor cells in each ear. These are primary sensory cells with no intervening synapses. While pulsed tones trigger receptor and action potentials, continuous tones lead to rapid adaptation of spike activity leaving the receptor potential as the only bioelectric event (Adams and Belcher, J. Acoust. Soc. Am. 56:S40, 1974). This behavior presents an ideal system for biochemical studies of transduction, i.e., sound-induced generation of receptor potential. Results obtained from our species, Agrotis ypsilon, confirm previous electrophysiological findings from other Noctuids. The hearing organ is sensitive to ultrasonic frequencies (highest sensitivity at 40 to 60 kHz) and shows the above adaptation pattern. Biochemical studies demonstrate that this organ is amenable to radiotracer studies. Typically, 5 µl of carrier-free ^{32}P-orthophosphate are injected into the moth's thorax. Moths are sacrificed by injection of glutaraldehyde followed by microwave irradiation. Labeled ATP, phosphoproteins, and phospholipids are measured in the nodular sclerite, a thick strip of cuticle adjacent to the tympanic membrane, and in the scoloparium where the two sensory cells are located. The time course of phospholipid labeling is very similar to that of mammalian inner ear tissues: polyphosphoinositides (phosphatidyl inositol phosphate and diphosphate) are the most highly labeled lipids reaching maximal labeling in 30 min. Phosphatidic acid incorporates less ^{32}P but shows a similar time course while phosphatidyl inositol and ethanolamine have a much slower rate of ^{32}P-incorporation. The polyphosphoinositides are of special interest for an investigation of transduction because their rapid metabolism has been implicated in the control of membrane permeability possibly via binding and release of calcium. Also, their turnover has been shown to be affected by ototoxic drugs. (Supported by grants from The Deafness Research Foundation, the Michigan Memorial-Phoenix Project, and by Program Project Grant NS 05785).

685 MONOSACCHARIDE TRANSPORT IN CAPILLARIES ISOLATED FROM RAT BRAIN. Alan R. Kolber* and Pierre Morell. Biol. Sci. Res. Ctr., Dept. Biochem., Univ. North Carolina Med. Sch., Chapel Hill, N.C. 27514

We have developed a rapid method for the isolation of rat brain microvessels in high yield, and have initiated a kinetic study of monosaccharide uptake by these capillaries.

Pooled whole brains of 25-day old Sprague-Dawley rats were minced and then homogenized in a phosphate-buffered Krebs-Ringer medium containing 0.5% polyvinyl pyrollidine (PVP-Ringer). Material passing through a 351 µ nylon screen, but retained on a 44 µ screen, was suspended in 1.8 M sucrose in PVP-Ringer; and these capillaries were further purified by floatation ultracentrifugation in a 1.8 M-1.2 M-sucrose discontinuous gradient in PVP-Ringer. The capillary fraction contained about 0.5% of the total brain homogenate protein.

Monosaccharide uptake experiments were performed in a 30 µl total incubation volume containing 75 µg protein and 1 µCi of [^{3}H]sugar in PVP-Ringer. The reaction mixtures were incubated for various lengths of time at 30°C and the reactions were terminated by addition of 3 ml ice-cold PVP-Ringer and collection of the capillaries on a Millipore filter. The filters were washed, dried, and the radioactivity determined by liquid scintillation counting.

The kinetic parameters examined to date suggest that monosaccharide uptake by isolated rat brain capillaries fulfills the following criteria for "carrier-mediated" diffusion: (1), 3-O-methylglucose (3-OMG) uptake into isolated capillaries exhibits saturation kinetics — the half-times of uptake for several concentrations of this sugar vary more than predicted on the basis of simple diffusion; (2), kinetic analysis of stereospecific inhibition of 3-OMG uptake by 2-deoxy-D-glucose indicates that this sugar has a higher affinity for transport than 3-OMG; (3), 3-OMG uptake is inhibited by the glucoside, phloridzen, a specific inhibitor of carrier-mediated monosaccharide transport; (4), isotope distribution data suggests that 3-OMG distributes in the available capillary (cell) water space to a concentration not exceeding that in the external medium — the uptake is non-concentrative.

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686 CHOLESTEROL CONTENT MODULATES THIOPENTAL'S MEMBRANE/BUFFER PARTITION COEFFICIENT. K. Korten* and K.W. Miller*. (SPON: E.T. Hedley-Whyte). Harvard Medical School, Depts. Anaes. & Pharm., Peter Bent Brigham and Massachusetts General Hospitals, Boston, Massachusetts.

General anesthetics have long been thought to act by a mechanism involving non-specific dissolution into the hydrophobic regions of membranes, yet the role that membrane composition plays in modulating the amount of anesthetic adsorbed, and the possible relation of this to the specificity of anesthetic action, has not been systematically explored. We report here membrane/buffer partition coefficients (λ) of thiopental in a number of biological membranes and in artificial membranes of defined lipid composition.

λ was determined at 25°C and pH 6.6 using ^{35}S-thiopental. After equilibration suspensions of human erythrocyte ghosts, rat liver mitochondria or microsomes were sedimented by centrifugation and samples were taken from both the supernatant and pellet for scintillation counting. Red blood cell membranes were also depleted of cholesterol during a preincubation with sonicated phosphatidylcholine vesicles, from which they were subsequently separated by centrifugation. After washing and resuspension they were equilibrated with ^{35}S-thiopental and λ determined as above. The protein, phospholipid and cholesterol contents of all biomembranes were determined spectrophotometrically. The λ of thiopental in sonicated phospholipid bilayers, which are not readily sedimented, was determined by ultrafiltration and scintillation counting of the lipid suspension and of the filtrate.

In erythrocyte ghosts λ was found to be 25, but rose to 40 after partial cholesterol depletion. In mitochondria and microsomes λ was found to be 47 and 77 respectively.

The intact biomembranes present a heterogeneous hydrophobic medium. Our data allow some conclusions to be drawn about the distribution of the anesthetic in the membrane. No simple correlation was observed between λ and the proportion of protein in the membrane, so that a relatively small fraction of the anesthetic is probably associated with protein. On the other hand λ increased as the cholesterol/phospholipid ratio decreased in both the biomembranes and the lipid bilayers of defined compositions, suggesting that most of the anesthetic is dissolved in the lipid region. Compared on this basis only λ for the microsomal membranes deviated somewhat from that expected from its cholesterol content. Thus the cholesterol content of biomembranes appears to be the most important, but not the only, factor determining thiopental's partition coefficient. (Supported by USPH Grant GM-14904).

687 POTASSIUM AND CALCIUM ION CONDUCTANCE NOISE IN HELIX NEURONS. K.S. Lee*, N. Akaike*, A.M. Brown, H.M. Fishman and L.E. Moore. Dept. Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Voltage-dependent unit channel conductances, presumed to be the basic units of membrane excitability, have been measured by fluctuation analysis of membrane noise observed in squid axon, node of Ranvier and frog neuromuscular junction. The unit conductances have values of 10^{-11} to 10^{-12} mhos. Similar measurements have not been made in nerve cell bodies and would be of interest since somas have larger specific membrane resistances and more numerous ionic conductances than axons. We made these measurements on isolated Helix aspersa nerve somas using a method which combines voltage clamp with internal perfusion. Membrane noise increased by 2-4 orders when membrane potential was stepped from -60 to -10 mV. Specific K^+ ion conductance noise was determined by computing difference power spectral densities from individual neurons before and after substituting Cs^+ for K^+ intra- and extracellularly. Under these conditions Cs^+ suppresses K^+ currents. Power density spectra of current noise measured during a 15 sec voltage clamp step to -10 mV were calculated using a Fast Fourier Transform algorithm. At these long times outward K^+ current is steady and the transient K^+ current is inactivated. Potassium difference spectra were fitted to a single Lorentzian function by a Marquardt least squares method. A unit channel conductance, $\gamma_K \cong 10^{-14}$ mhos, was calculated using the relationship $\gamma = \sigma^2/\bar{G}(1-a)$ where σ^2 is the area under the fitted Lorentzian curve, $\bar{G}$ is the mean conductance and a is the ratio of the conductance at a given potential to its maximum value. Similar procedures were employed for the calcium conductance (G_{Ca}) using nickel ions to suppress G_{Ca}. The unit Ca^{++}conductance was $\gamma_{Ca} = 10^{-15}$ mhos. The $K^+$and Ca^{++} noise spectra had corner frequencies of ~20 Hz consistent with voltage clamp relaxation times measured at these potentials. The small unit conductance values found in these experiments, as compared to those found in other neural preparations, suggest that some process other than free diffusion in aqueous pores is responsible for ion movements in the soma membrane.

688 EFFECT OF EXTRACELLULAR POTASSIUM ON THE STEADY-STATE ELECTROGENIC Na-K TRANSPORT SYSTEM OF THE CRAYFISH GIANT AXON. Edward M. Lieberman. Dept. Physiol., Sch. Med., East Carolina Univ., Greenville, North Carolina 27834.

A previous investigation from this laboratory (Lieberman & Nosek. Pflugers Archiv. Europ. J. Physiol. 366:195, 1976) demonstrated that the crayfish axon electrogenic transport system contributes approx. -7mV to the total steady-state membrane potential of -85mV. Electrogenic current is approx. 3.5μA/cm^2. The Na to K transport ratio was calculated from a modification of the conductance equation for non-equilibrium potential and ionic steady-state using electrical parameters measured with space and current clamp techniques. The Na/K transport ratio ranged between 1.7/1 and 2.1/1 for control steady-state conditions.

The investigation has been extended to the effects of extracellular K on the electrogenic potential, current and transport ratio. The axial wire space and constant current clamp methodology has been previously described (Lieberman and Lane. Pflugers Archiv. Europ. J. Physiol. 366:189, 1976). All axons were equilibrated in Cl-free solutions using Isethionate as the anion substitute. Ouabain (5×10^{-4} to 10^{-3}M) was used to determine the electrogenic transport contribution to the membrane potential. Ouabain poisoning caused no change in membrane resistance except that directly attributed to the change in membrane potential.

$[K]_o$ between 0 and 21mM did not appear to effect $[K]_i$ for periods of up to 3 hours. $[Na]_i$ is minimally affected as indicated by little change in the AP overshoot and direct measurements of $[Na]_i$ (Wallin, Acta physiol. scand. 70:431, 1967). At each $[K]_o$, new potential steady-states were reached in 5-10 min. and remained stable for up to 3 hours. Net transport current was least at $0[K]_o$ (3μA/cm^2) and greatest at $21.6[K]_o$ (7μA/cm^2). The contribution of the electrogenic transport system to membrane potential was greatest at $0[K]_o$ (14 mV) and least at $21.6[K]_o$ (6 mV) as a result of membrane resistance changes which occur with external K. Evidence suggests that the crayfish axon transport system is able to adjust to changes in $[K]_o$ to maintain intracellular ionic constancy for periods of up to 3 hours. Under these circumstances the assumption that a steady-state exists seems valid and the modified steady-state conductance equation may be used to calculate ionic currents, conductances and the Na/K transport ratio. The findings of this study indicate that the transport ratio is variable and ranges from 1.25/1 at nominally $0[K]_o$ to extremely high values (28/1) at 16.2 $[K]_o$. Project supported by NIH grants NS08773 and NS13531.

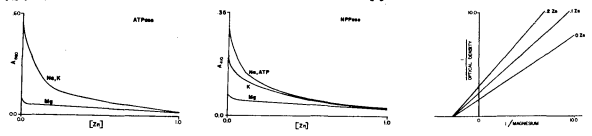
689 ACTIONS AND INTERACTIONS OF ZINC ON RAT BRAIN NA,K-ATPASE AND A PARTIAL REACTION. G.L. Longenecker, H.E. Longenecker, Jr. and L.J. Kopaciewicz*. Depts. Pharmacol./Physiol., U. So. AL Med. Coll., Mobile, AL 36688

We have shown effects of Zn at low concentration (>10uM) on frog neuromuscular junction (NMJ) which resemble effects of Na,K-ATPase inhibitors (e.g., ouabain). Attempts to correlate symptoms of Zn poisoning with effects on Na,K-ATPase are variable: some show Zn can substitute for magnesium (Mg) and support Na,K activation, others that it inhibits by competition with ATP. We have examined the effects of Zn on a heavy microsomal Na,K-ATPase, and on a partial reaction.

Na,K-ATPase was prepared from homogenates of stripped rat brains in .25M sucrose buffered with histidine, containing disodium EDTA in all but the final solution. Assay mixtures contained 40 mM tris (pH 7.4), 2mM ATP, 3mM Mg, 100mM Na and 10mM K. Reactions were started by addition of ATP, and followed by Pi determination. The enzyme hydrolyzed ATP minimally in the presence of Mg alone, with a ouabain-sensitive 5-6x increase on addition of Na/K. The K-sensitive dephosphorylation step was examined by use of p-nitrophenylphosphate (NPP). Assay conditions were 40mM tris (pH 7.4), 4mM Mg, mM K, 10mM Na and mM ATP. Reactions were started with NPP. Yellow color of p-nitrophenol was read at 410nm. NPPase activity was minimal with Mg, increased with K, and increased further with Na and ATP. Zn as the acetate was preincubated with the enzyme in both assays.

Zn inhibited both the Na,K- and Mg- components of the ATPase; the I-50 (concentration for 50% inhibition) of the Na,K component was c 100uM graphically (fig 1). All components of NPPase were inhibited with I-50s for the K- and Na,ATP- components also c 100uM (fig 2). We have shown non-competitive inhibition between Zn and Mg (fig 3).

Zn inhibition of Na,K-ATPase is consistent with effects of Zn at the NMJ. The concentrations necessary to inhibit Na,K-ATPase correlate with those affecting the NMJ: the lower the Zn, the longer the time for NMJ effects to develop. Zn affects Na,K-ATPase and NPPase by non-competitive inhibition between Zn and Mg. Additional effects of Zn at the NMJ include a Mg-like inhibition of stimulated transmitter release, i.e., calcium antagonism: this may also occur via a binding of Zn to ATPase or other Mg sites. (U.S.A. Intramural/NIH 1R01ES01321-01 support)



690 **TOPOGRAPHICAL STUDIES OF GLYCOPROTEINS AND GANGLIOSIDES IN SYNAPTOSOMES.** S.P. Mahadik*, B. Hungund*, and M. M. Rapport, (SPON: H. Tamir). N.Y.State Psychiatric Inst., New York 10032.

We have studied synaptosomes by the method of Steck (1972): i.e. oxidation with galactose oxidase followed by reduction with [^{3}H]-Na borohydride, which labels exposed terminal galactose and galactosamine residues of glycoproteins and glycolipids. Purified synaptosomes were labeled and disrupted by osmotic shock; the membranes were then fractionated on a step gradient of diatrizoate (Tamir et al., Anal. Biochem., 1976) to give 4 membrane fractions (A to D) and a mitochondrial pellet (E). Specific incorporations (ratio of label found with enzyme to that without enzyme) were: A, 4.5; B, 2.4; C,D,E, $\leq$ 1.6. When membrane fractions were first isolated and then labeled, specific incorporation was 5 to 6 fold greater but showed little discrimination among fractions. Membrane fractions A and B obtained from labeled synaptosomes contained 1/3 of the label in protein and 2/3 in lipid. After labeling isolated membranes, the label was $<$ 1/5 in protein and $>$ 4/5 in lipid. Since the highest degree of specific labeling after treatment of intact synaptosomes was found in fractions A and B, and since this and other evidence indicate that these are the purest synaptic plasma membrane fractions, their protein and ganglioside components were analyzed. Protein from these delipidated membranes showed 6 labeled bands in 10% polyacrylamide tube gels with M_r of 96K, 72K, 53K, 33K, 25K, and $<$ 17K daltons. After labeling the isolated membranes, the labeled bands were M_r 130K, 96K, 80K, a broad region from 70K to 50K, 34K, 27K, and $<$17K. Labeled bands were PAS positive. The highest degree of staining and label incorporation were in the 70K to 50K region. Polypeptides in gradient slab gels showed large incorporation of label in three regions: 160K to 96K, 70K to 40K, and 7.8K to 3.2K. These results show that most of the polypeptides in the ranges 160K to 96K and 70K to 40K were not labeled in intact synaptosomes but became accessible to the enzyme in isolated membranes. The polypeptides that predominate on the external synaptosome surface are the 70K band and the smaller polypeptides in the range of 7.8K to 3.2K. Most of the specific label in the lipid fraction after labeling intact synaptosomes was present in G_{M1} ganglioside; its presence in the galactose residue was confirmed. After labeling isolated membranes, the label was found in zones corresponding to G_{M2}(45%), G_{M1}(26%), G_{D2}(15%), G_{D1b}(14%). Since the carbohydrate-containing molecules in the membrane are considered to be associated with the outer surface and since only a small portion of these molecules have their saccharide chains exposed in the intact synaptosome, the molecular organization in the membrane must be very close-knit. After disruption, this tight organization appears to fall apart with consequent exposure of most of the constituent saccharide chains.

691 ASSOCIATION OF ^{3}H-ALPRENOLOL WITH MEMBRANES: LOCAL ANAESTHETIC AND β-ADRENERGIC EFFECTS. Carl Mendel* and Richard R. Almon. School of Med., Univ. of Calif, San Diego and Division of Cell and Mol. Biology, State University of New York at Buffalo, Buffalo New York 14214.

The conceptual relationship between displaceable ligand associations and "specific binding" to receptors is an integral part of the current approach to studying populations of receptor sites. The present report examines this relationship with respect to the study of putative β-ADRENERGIC receptor sites. The antagonists alprenolol, propranolol (Pro) and pindolol all have considerable local anaesthetic effects which are related to the partitioning of these compounds into cell membranes. As an approach to distinguishing partitioning from binding, a comparative analysis was made of the association of (-) ^{3}H-dihydroalprenolol (^{3}H-Alp) with human erythrocyte (RBC) membranes and with membranes derived from brain. The assumption was that the human RBC membranes would exhibit only displaced partitioning, whereas the membranes from brain would exhibit both displaced PARTITIONING and BINDING. The data show that the ^{3}H-Alp partitions into biological membranes. The addition of other agents such as Pro which also partition into the membranes, alters the membrane to a sufficient degree such that the ^{3}H-Alp partition coefficient changes. If the partition coefficient is lowered as is the case when Pro is added, the ^{3}H-Alp is displaced from the membrane. The ability of "competing agents" to change the ^{3}H-Alp partition coefficient is related to that compound's n-octanol-water partition coefficient and its ability to act as a local ANAESTHETIC. In addition to its ability to mimic displaceable surface binding, the partitioning also can influence adenylate cyclase activity. Membranes from brain tissue do contain another class of associations not present in human RBC membranes. Unlike the partitioning (present in both) this class of association has all the characteristics expected of the association of ^{3}H-Alp interacting with a single set of identical independent sites with a Ka of 2.04 x $10^8 M^{-1}$. It is concluded that the use of ligand displacement to define putative β-adrenergic receptor population is NOT ENTIRELY VALID because 1) it assumes all displaceable ligand is bound to unique surface structural sites; 2) it does not consider the possible equilibrium distribution of the radioactive ligand among several different environments; 3) it does not consider the possibility that the displacing agent may also distribute among the same several micro-environments and consequently change their properties and 4) that this change in the properties of particular micro-environments may alter not only the labeled ligand partition coefficient, but also the activity of secondary transduction systems such as adenylate cyclase.

692 SURFACE PROTEINS OF DIFFERENTIATING RAT CEREBELLAR CELLS MAINTAINED IN DISPERSED CELL CULTURE. Lee N. Minier* and Robert S. Lasher. Dept. Anatomy, Univ. Colo. Med. Center, Denver, Colorado 80262.

Several lines of evidence suggest that developmental processes may be partially mediated through varying arrays of cell surface components (proteins, glycoproteins) during growth and differentiation of given cell types. Using enzymic radioiodination as a cell surface probe, neuronal and non-neuronal cells of rat cerebellum maintained in dispersed cell culture were evaluated for their complement of cell surface proteins during differentiation in vitro. Particular attention was given to the appearance and distribution of the large, external, transformation-sensitive (LETS) protein. Neuronal and non-neuronal cell types were freed from the culture surface with the aid of an electrolytically sharpened tungsten needle and collected in a micropipette attached to a micromanipulator. Initial solubilization of the surface proteins was done in a lysing buffer containing urea, 2-mercaptoethanol, the non-ionic detergent Nonidet P 40 and water. Use of this buffer facilitated removal of DNA from the sample by subsequent centrifugation at a force of 10,000 x g. Final solubilization of membrane-associated proteins was accomplished by addition of the sample to an electrophoretic buffer containing sodium dodecyl sulfate as a primary chaotropic agent. These surface proteins were resolved by microelectrophoretic, radioautographic and fluorographic methods. Neuronal cells from various postnatal age rat cerebellums maintained for different periods of time in vitro were found to have very little or no iodinatable LETS protein on their surfaces. Conversely, non-neuronal cells associated with cerebellum were found to have significant amounts of LETS on their surface at all times of growth in vitro investigated. This protein apparently is the major iodinated surface component of non-neuronal cells, and co-migrates with purified LETS protein from chinese hamster ovary cells. In addition, non-neuronal cerebellar cells transformed by SV40 virus appeared to have reduced levels of LETS protein on their surfaces.

Other variations in the surface protein electrophoretic patterns of neuronal and non-neuronal cell types are presented and discussed with reference to potential age-dependent alterations of cell surface organization. Such alterations appear to be generally restricted to the low molecular weight classes of peptides. Research support: NIH Training Grant GM 01981 (LNM) and NIH Grant NS13133 (RSL).

693 ELECTROPHYSIOLOGICAL AND GENETIC DISSECTION OF THE MEMBRANE OF PARAMECIUM. Donata Oertel, Stanley J. Schein* and Ching Kung*. Lab. Mol. Biol. and Dept. of Genetics, Univ. Wisc., Madison, WI 53706.

In order to understand better the molecular biology of electrical excitability in biological membranes, mutants have been isolated with genetically altered membranes. Voltage clamp studies show that Paramecium tetraurelia has at least 3 voltage dependent conductances which presumably correspond to 3 kinds of membrane channels: 1) a calcium conductance sensitive to depolarization, 2) a rectifying conductance sensitive to depolarization, and 3) a potassium conductance sensitive to hyperpolarization. Step depolarizations of the Paramecium membrane result in a transient inward current and then a steady state outward current. The calcium inward current and rectifying outward current were separated by ion substitution and by using a mutant which lacks functional Ca^{++} channels. This showed that Ca^{++} channels inactivate. The difference in voltage sensitivity enables us to distinguish the hyperpolarization-sensitive K^+ channels from depolarization-sensitive channels. Reversal potentials of "tail" currents in ionic environments with various K^+ concentrations obey the Nernst relation showing that these channels are specific for K^+.

694 VOLTAGE CLAMP STUDIES OF INTERACTIONS BETWEEN HALOTHANE AND HIGH HYDROSTATIC PRESSURE ON APLYSIA BURSTING NEURONS. J. Farmentier, P.B. Bennett* and B.B. Shrivastav*. Dept. of Anesthesiology, Duke University Medical Center, Durham, North Carolina, 27710

Exposure to halogenated hydrocarbon anesthetics initially increases the firing rate of autoactive neurons in the Aplysia CNS but after longer contact blocks this activity. Bursting neurons show an initial increase in the frequency of membrane oscillations, with more impulses per burst, followed by gradual membrane hyperpolarization and loss of activity. During early stages of narcosis depolarizing current can trigger action potentials of waveform and duration similar to normal impulses, which implies that mechanisms controlling these events are not seriously disrupted while currents controlling the slow waves are altered.

Identified bursting neurons (L3, L6, R15) were voltage clamped inside a pressure chamber with a single microelectrode clamp system (Wilson and Goldner, 1975, J. Neurobiol. 6:411-422) and complete current-voltage (I-V) curves were recorded using 5 second long voltage steps to identify the negative resistance region (-50 mV to -30 mV) which is responsible for slow wave activity. The pressure chamber was then filled with mineral oil, for the compression experiments, or mineral oil in which a sufficient amount of halothane had been dissolved to equilibrate the bathing medium of the ganglion to a calculated percentage of saturation.

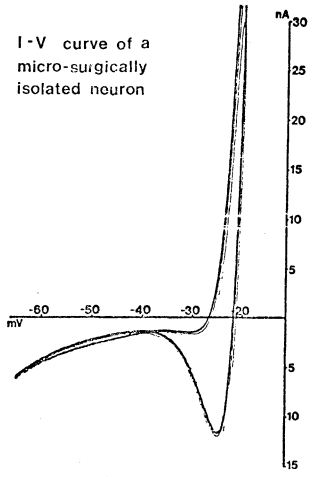
Exposure to halothane at 3-5% of saturation results in gradual (15-45 minutes) loss of the negative resistance characteristic (NRC) of the membrane until the I-V plot becomes linear. The long time delay we attribute to the large size of the Aplysia neurons. A plot of current at the peak voltage of the NRC against exposure time is a monitor of the developing anesthetic effect. Exposure to higher than 6% saturation results in a sudden reduction of current required to clamp the cell throughout its voltage range, followed by the above mentioned shift to near ohmic characteristics of the negative resistance region.

Hydraulic compression to 4000 p.s.i. results in a reduction of the holding current in the negative resistance region but the NRC is not eliminated. Compression to 4000 p.s.i. after sufficient exposure of halothane to result in linearization of the curve does not restore the NRC, and compression immediately following exposure to halothane does not prevent the anesthetic effect from developing. These results suggest that pressure reversal may be a multi-neuronal phenomenon and thus of a different nature than the direct effects of either pressure or anesthetics on active membrane processes. This work was supported by a Departmental Sponsored Award from the Dept. of Anesthesiology, Duke University Medical Center.

695 SEMI-STATIONARY CURRENT VOLTAGE CHARACTERISTIC OF REPETITIVELY FIRING NEURONS. L. Donald Partridge, Stuart H. Thompson*, Steven J. Smith*, and John A. Connor*. Friday Harbor Labs., U. of Wash., Friday Harbor, Wash., 98250.

The I-V curves of repetitively firing (non-bursting) neurons show non-linearities in the sub-threshold region. Micro-surgically isolated molluscan neurons were studied under voltage clamp during slow ramp voltage changes and were found to exhibit a region of negative slope conductance and a current hysteresis between the depolarizing and hyperpolarizing directed ramps. These I-V characteristics are especially important in their relationship to repetitive firing as they occur in the voltage region near spike threshold.

Ion substitution experiments suggest that the negative slope characteristic results from a non-inactivating or slowly inactivating Na^+ current with some further contribution of Ca^{++} ions. The hysteresis is a semi-stationary characteristic that becomes less pronounced as the rate of change of voltage is decreased. Injection of EGTA into the cell or replacement of Ca^{++} with Ba^{++} in the bathing solution reduces or abolishes the hysteresis suggesting a role of Ca^{++} in the phenomenon. It is thought that the hysteresis results from a Ca^{++} activated K^+ current.

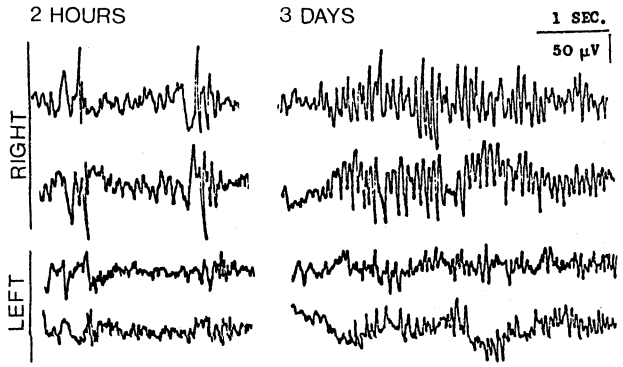


696 VOLTAGE-DEPENDENT CALCIUM CONDUCTANCE IN RODS. F. N. Quandt*, G.L. Fain*, and H.L. Gerschenfeld* (SPON: L.Kruger). UCLA Sch. Med., Los Angeles CA 90024, and Ecole Normale Superieure, Paris, France.

The membrane of rods is usually assumed not to have voltage-dependent conductances. The effects of compounds, known to block specific voltage-dependent conductances in other membranes, were determined in rods in order to examine more carefully the possible presence of these conductances. In the superfused, isolated retina of Bufo marinus, 6-12 mM tetraethylammonium (TEA) added to normal ringer produces a 5 mV depolarization of the rod membrane. Receptor potentials in TEA show a faster decay and are followed by a 3-4 Hz oscillation of the membrane potential. The peak-to-peak amplitude of these oscillations is initially as large as 35 mV but decays to zero within 5 sec. Similar oscillations are obtained with the addition of 4-aminopyridine to ringer, or by replacing Ca^{2+} with Sr^{2+} or Ba^{2+}. Ca^{2+} appears to be the major current carrier involved in the production of regenerative potentials during exposure to TEA. The amplitude of the TEA-induced oscillations decreases with decreasing external Ca^{2+}. Increasing external Ca^{2+} produces regenerative potentials which resemble action potentials and are spontaneous in the dark. The depolarization phase of the action potentials is up to 45 mV in amplitude, and 60-80 msec in duration. A subsequent 5 mV hyperpolarization phase decays in .5-1 sec. Sr^{2+} substitutes for Ca^{2+} in producing these action potentials as the peak amplitude increases with increasing Sr^{2+}. Action potentials can be recorded in a zero Na^+ringer with 28 mM Sr^{2+} and TEA. The TEA-induced oscillations in normal Ca^{2+} medium are reversibly blocked by the addition of .1 mM Co^{2+}, 5 mM Mg^{2+}, or .1 mM D-600; but not by $2 \cdot 10^{-6}$ M TTX. The oscillations are not due to an interaction between rods and horizontal cells since 2-5 mM Na^+ aspartate, which blocks the responses of horizontal cells, has no effect even though the simultaneously recorded b-wave of the e.r.g. is eliminated. The rod membrane must contain a voltage-dependent Ca^{2+} conductance which can generate regenerative potentials in the presence of TEA. TEA may act by partially blocking a voltage-dependent K^+ conductance. This potassium conductance could normally prevent the Ca^{2+} conductance from generating these regenerative potentials.

697 CHOLERA TOXIN INDUCES EPILEPTIFORM ACTIVITY. Rapport, M.M. and Karpiak, S.E. Div. Neurosci. NY State Psychiatric Inst. & Dept. Biochem., Columbia U., Coll. P & S, New York, N.Y., 10032.

The action of cholera toxin involves the combining of its B subunits with a specific G_{M1} ganglioside receptor on the cell membrane followed by penetration of its A subunit into the cell causing activation of adenylate cyclase (Sahyoun & Cuatrecasas, PNAS, 1975; Fishman & Brady, Science, 1976). Since G_{M1} ganglioside receptors are involved in the induction of recurrent epileptiform activity by antibodies (Karpiak, Graf & Rapport, Science, 1976), it was of interest to determine whether cholera toxin would, under similar conditions, also induce seizure activity. Rats were injected intracortically (∠2mm into the right sensorimotor cortex with 1 µg of purified cholera toxin in 10 µl Tris buffer (pH 7.5). The animals were then implanted with 6 cortical screw electrodes into the calvarium. The electrodes were attached to a microamphenol plug and affixed to the skull with acrylic cement. EEG recordings were a) taken from alert animals 15 min after surgery b) monitored for several hours c) then taken daily. The cholera toxin caused all rats to develop spiking activity ipsilaterally in 1 to 3 hrs (Fig). This activity increased in frequency on subsequent days, with some contralateral spread, and lasted for more than two weeks. This effect of cholera toxin provides additional evidence for the involvement of G_{M1} ganglioside receptors in the induction of seizure activity.



EEG 3 hrs and 3 days after injection (RIGHT) of cholera toxin.

698 CHANGES IN CONDUCTION VELOCITY ACCOMPANY ACTIVITY-DEPENDENT SHIFTS IN THRESHOLD OF FROG SCIATIC AXONS. Stephen A. Raymond. Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

Conduction velocity in an axon is related to its threshold level. This study has investigated the extent of this correlation throughout each of the phases of threshold oscillation now known to follow conditioning impulse patterns in frog nerve fibers. Conduction velocity can be influenced over the entire course of an axon by changes in ionic milieu, currents generated in nearby structures, and shifts of local metabolism. Therefore, to assay conduction velocity changes arising solely from impulses traveling in the axon itself, extracted sciatic nerve fibers bathed in recirculated Bolye-Conway Ringer's at constant temperature, and pH were used.

The nerve trunk was stimulated with monopolar current pulses varying from 50-300μsec in duration. Threshold was hunted by adjusting the stimulus duration so that a dissected single fiber recorded by suction electrode at the end of the nerve responded to 50% of the stimuli. Conduction velocity was measured using a counter gated by the leading edge of the stimulus and closed by the arrival of the response at the recording electrode.

During the relatively refractory period conduction velocity showed a steep speed up corresponding to recovery of threshold. Throughout this period impulses are slower than those in the rested fiber. Threshold crossed the resting level entering the supernormal phase just as the velocity curve crossed its resting value. Delays to crossover match within 50μsec. Peak supernormality coincides with peak conduction velocity and the slow increase of threshold after the peak is matched by a diminishing velocity. The crossover from supernormality to depression also matches even as the crossover is made to occur earlier after conduction pulses by increasing depression. Velocity is slowed throughout depression by an amount proportional to the threshold level. Changes of up to 20% in conduction velocity often parallel a 50% increase in threshold. Slow fibers showed greater changes of conduction velocity during all phases than faster ones.

During intermittent conduction the velocity slowed progressively during the conduction periods. It reached a minimal level just before conduction failed. When conduction resumed, the initial impulses traveled as fast as in rested nerve. Then velocity slowed as depression built up. These observations are consistent with an explanation of intermittent conduction in terms of threshold changes.

The strict correlation between threshold and conduction velocity changes observed in controlled conditions on excised nerve fibers suggests that the same membrane processes and mechanisms underlie both phenomena. Both can be used to measure activity dependence of membrane processes on impulse conduction.

699 INWARD Ca CURRENT IN FROG SKELETAL MUSCLE FIBERS. J. A. Sánchez, L. Nicola Siri* and E. Stefani. Depto. de Fisiología y Biofísica, Centro de Investigación del I.P.N., Apdo. Postal 14-740, México 14, D.F.

It was recently described in frog skeletal muscle fibers a calcium dependent slow spike which was found by removing chloride and blocking potassium currents. In the present experiments we studied the membrane current underlying the slow calcium spike with voltage clamp using the three microelectrode technique. The saline contained: 40 mM Tetraethylamonium (TEA), 40 mM Na, 2.5 mM K, 10 mM Ca, 113 mM sucrose, sulfate as the anion and was buffered at pH 7.3. Mechanical artifacts were avoided by rolling and stretching the frog sartorius muscle around a 2 mm diameter perspex rod. On occasions, 350 mM sucrose was added to the saline. The experiments were performed at room temperature (19-23°C). Using long depolarizing voltage pulses (500 msec), there was an initial outward current due to the leak current and a remaining potassium current which was followed by a transient inward current. The latency and amplitude of the inward current depended upon the voltage pulse amplitude and it had a threshold of -25 mV. The latency measured at the maximum negative dI/dt was 400 mseg for threshold pulses decreasing up to 50 msec for large pulses. When increasing pulse amplitude, the potassium outward current increased in spite of TEA, and the inward current could not be accurately measured. This was partially overcome by extrapolating the potassium current which was substracted from the total current. The inward current was 114 + 24 $\mu A/cm^2$ (14, mean + SE) at ca 0 mV and the range of the extrapolated equilibrium potential was between +60 and +100 mV. The inward current was blocked by Co (10 mM), but not by tetrodotoxine (10^{-6}M). Furthermore, it was not sodium-dependent and it was suppressed by removing external calcium. These results indicate that calcium is the major ion contributing to this current. This calcium current inactivates. With prepulses of 500 msec the inactivation curve follows the expression: $h = 1/(1 + \exp((V - Vh)/k))$ with V_h of -23mV and k 6mV. This calcium channel resembles the slow calcium channel of the squid axon membrane. Its probable role related to the excitation contraction coupling mechanism will be discussed.

700 CALCIUM SPIKES IN HIPPOCAMPAL PYRAMIDAL CELLS. Philip A. Schwartzkroin and Mara Slawsky*. Dept. Neurol., Stanford Univ. Sch. Med., Stanford, CA 94305.

Using an in vitro hippocampal slice preparation, we have shown that CA1 pyramidal cells can generate a calcium spike. Slices 350-400 μ thick were cut from guinea pig hippocampus and incubated as previously described. Intracellular recordings were obtained by introducing potassium acetate micropipettes into CA1 cell body layer, stratum pyramidale. The cell body layer was visible as a light band on the trans-illuminated slice and clearly differentiable from the dendritic regions. Monopolar stimulation in stratum radiatum (Schaffer collaterals) evoked synaptic potentials and spikes; stimulation in the alveus elicited antidromic spikes. Intracellular depolarizing current pulses elicited trains of action potentials, with current threshold for spike firing about 0.25 nA. During an intracellular penetration, tetrodotoxin (TTX) (10^{-4} M) could be applied to localized regions of the slice by pressure ejection from a broken micropipette. Small droplets of TTX deposited at the cell body-basal dendrite region abolished antidromic spikes and spiking evoked by the usual depolarizing current pulses, but left intact synaptically elicited slow potentials; application of TTX to the apical dendritic region blocked synaptic potentials. When sufficient TTX had been applied to abolish all normal spiking activity in a cell, intense (1.5-4.0 nA) intracellular depolarizing pulses evoked long duration (50-60msec), slowly rising and falling, all-or-none "spikes". Application of droplets of calcium chloride (20mM) to the CA1 region (particularly in the apical dendrites) potentiated the amplitude of the TTX-resistant potential and made it much sharper (more spike-like). Manganese ion effects were then tested, since Mn^{++} is known to block Ca^{++} currents. Droplets of manganous chloride (20mM) deposited at the same sites as the Ca^{++} completely blocked elicitation of the TTX-resistant spike, or significantly raised the current threshold for spike initiation. Barium, an ion which can carry calcium currents as well as decrease some component of the potassium conductance, had complex effects when applied to the TTX-treated slice. Initially 20mM Ba^{++} caused a cell depolarization and spontaneous firing of long-duration spikes which were similar in appearance to cardiac action potentials. Low intensity depolarizing pulses (<1.0 nA) were sufficient to trigger all-or-none long lasting potentials. All drug effects were reversible, with normal cellular potentials returning within an hour of drug application. Drug effects were also localized, so that regions of a given slice adjacent to that receiving drug droplets displayed normal spiking activity. (Supported by grant NS 12151 from the NINCDS, NIH.)

701 MODULATION OF ELECTRICAL ACTIVITY IN APLYSIA NEURONS BY CHOLESTEROL. Cathy L. Stephens and Meir Shinitzky*.

Electrical activity of a single neuron from Aplysia californica was recorded while perfusing an isolated ganglion with lipids to modulate the membrane microviscosity. Sonicated liposomes of either egg lecithin (16 mg/ml) or egg lecithin (16 mg/ml) plus cholesterol (10 mg/ml) were diluted 1:4 (V/V) in sea water. The liposome-sea water mixtures were then applied to the ganglion during continuous electrical recording for up to 3 hrs. Perfusion of pacemaker and burster type neurons with lecithin-cholesterol liposomes, which increased the cholesterol level of the cell membrane, progressively diminished spontaneous activity and increased the threshold to elicit an action potential following intracellular stimulation. After 30-60 mins. incubation, the action potential was completely blocked, but the resting membrane conductance was unaltered. The spontaneous electrical activity of the cholesterol enriched neurons could be completely restored upon consecutive perfusion with lecithin liposomes for less than 30 minutes. This treatment, which presumably depletes the membrane cholesterol, demonstrated that the observed effect of cholesterol is reversible and passive. Furthermore, perfusion with lecithin liposomes of Aplysia neurons which were initially silent, generated continuous action potentials similar to pacemaker activity. These results strongly suggest that the activity of membrane sites which are involved in spontaneous electrical activity and generation of action potential is dependent on the membrane fluidity. Since the microviscosity of neural membranes may vary under various physiological conditions, such changes could induce significant alternation of neural activity in vivo.

702 IMMUNOCYTOCHEMISTRY OF BASIC PROTEIN IN CNS AND PNS MYELIN. N. Sternberger*, T. Tabira*, H. deF. Webster and M. W. Kies*. NINCDS, NIH, Bethesda, MD 20014.

Aldehyde fixed tissues from rat or Xenopus tadpole CNS and PNS were immunocytochemically stained with antibody to bovine myelin basic protein (BP) using the peroxidase-antiperoxidase method. Dense reaction product was observed on cut edges of myelin sheaths in tissue sections stained before embedding for electron microscopy. In isolated compact myelin, stained in suspension, reaction product was found only on outer lamellae or in centrally located areas where the lamellar structure was disrupted. Alcohol pretreatment of isolated myelin reduced the reaction with antibody to BP but enhanced the reaction with cerebroside antibody. The reaction of antibody to BP with thin sections of tissue stained after embedding was restricted mainly to the axonal and extracellular margins of intact myelin sheaths. No staining was observed after incubation with preimmune or BP absorbed sera.

703 DIFFUSION THROUGH THE SQUID AXON SCHWANN CELL LAYER. Robert E. Taylor, Francisco Bezanilla* and Eduardo Rojas* NINCDS,NIH, Bethesda,MD 20014; Dept. Physiology UCLA, Los Angeles, CA: Dept. Physiology, Univ.of East Anglia, Norwich, England.

We have investigated a number of models for diffusion through and resistance of the Schwann cell layer, taking the membranes of the Schwann cells to be impermeable. They are: 1) the anatomical model with the Frankenhaeuser-Hodgkin (F-H) space between the Schwann cells and the nerve membrane, the clefts between the Schwann cells, and the unstirred layer consisting of basement membrane, connective tissue and external solution; 2) anatomical model neglecting the convergence from the unstirred layer into the clefts but including specific and unspecific binding of saxitoxin (STX) and tetrodotoxin (TTX); 3) anatomical model without unstirred layer; and 4) thin membrane with unstirred layer.

These models were used to determine the conductance of a single sodium channel and the density of sites and kinetics of binding of TTX and STX (Keynes, et al. J.Gen.Physiol.61,267(1973); Bezanilla, et al. J. Gen. Physiol.61,268(1973); Keynes,et al. Phil.Trans.R.Soc. Lond.B.270,365-375(1975)).

We have considered the time course of F-H space concentration of: 1) potassium following initial loading; and 2) sodium, TTX or STX following sudden external concentration changes.

The effects of convergence into the clefts can be neglected both for diffusion and resistance. The clefts may be replaced by a thin membrane with small errors at short times. For sudden external changes in concentration the unstirred layer is always important. If the F-H space is initially loaded the unstirred layer may be neglected.

The thin membrane plus unstirred layer is the simplest adequate model which we have found for our studies of diffusion through the Schwann cell layer.

704 AXON LESIONS IN CHICK EMBRYO TIBIAL NERVES AFTER VINBLASTINE SULFATE (VBS). Betty Geren Uzman, Gloria M. Villegas* and Jeanne M. Curnutt*. VA Hospital and LSU Medical Center-Shreveport, LA 71130, and I.V.I.C., Caracas, Venezuela.

VBS injected into the chorio-allantoic (C-A) sac of 11 day incubated (dinc) chick embryos (C.E.) at a dose of 0.5μg VBS/g C.E. differentially affects bundle (B), segregated (S), isolated (I), and myelinating (M) fibers. As previously reported, 50% B fibers disappear 24-36 hours after VBS injection. Forty-eight hours after exposure to VBS, the axons of S, I, and M fibers exhibit a bull's eye appearance, with an electron-lucent (empty) zone between the axolemma and a central aggregate of neurofilaments, microtubules and granular material. Since S, I, and M fibers are generally affected when viewed in sizeable numbers in low-mag electron micrographs, and since their numbers are not significantly reduced after VBS injection, it is concluded that the "doughnut dissolution" and bull's eye aggregation of axoplasmic contents is consistent with fiber viability. In contrast, B fibers disappear in large numbers (≈ 20,-25,000) after VBS. Since the major difference between B fibers and S, I, or M fibers is the partial or complete envelopment of S, I, or M fibers by the Schwann cell, it is proposed that the apposition of axolemma to Schwann cell membrane is an unique junction stabilizing axolemmal (and axon) integrity against peripheral cytoplasmic (axoplasmic) disturbances of the cytoskeleton.

705 ALTERED ACTIVITY AND TEMPERATURE RESPONSE OF MEMBRANE-BOUND PROTEIN KINASES IN ERYTHROCYTES OF PATIENTS WITH MYOTONIC MUSCULAR DYSTROPHY. J.D. Vickers, M.P. Rathbone, and A.J. McComas, MRC Group in Developmental Neurobiology, Depts. of Neuroscience and Medicine, McMaster University Med. Centre, Hamilton, Ontario, Canada, L8S 4J9

Recently it has been demonstrated in erythrocytes from patients with myotonic dystrophy (MyD) that the phosphorylation of band 3 protein is reduced. We have examined the phosphorylation of spectrin, band 3 protein and phospholipids in erythrocyte membranes from MyD patients and healthy age- and sex-matched controls. We have also examined the temperature-dependence of these reactions. Using erythrocyte ghosts the incorporation of ^{32}P from [$\gamma^{32}P$] ATP into membrane proteins was determined at pH 7.4 at both 30°C and 37°C using the methods of Avrunch and Fairbanks (Biochem. (1974) 13:5507-5513). In 10 MyD patients incorporation of ^{32}P into band 3 protein was reduced at 37°C ($p<0.01$) was not different at 30°C from controls. Spectrin phosphorylation was reduced at both 30°C and 37°C compared to controls ($p<0.01$). Although phosphorylation of these membrane components was greater at 37°C than at 30°C in both controls and MyD patients the increase observed with patients was less than with controls (spectrin $p<0.025$, band 3 $p<0.001$). In two other patients (sisters) who had characteristic clinical manifestations of MyD, phosphorylation of both spectrin and band 3 protein was increased (> 2 standard deviations greater than values determined for other patients with MyD). Control values were comparable to controls for the other patients. In 5 out of 10 MyD patients the degree of phospholipid phosphorylation at both 30°C and 37°C was less than in controls ($p<0.001$). In the remaining 5 patients, phosphorylation was increased ($p<0.001$). Control values were similar for each group. Thus, altered phosphorylation of erythrocyte membrane components is not limited in MyD to a specific substrate. Although a primary genetic lesion in a single substrate protein has not been excluded, it is more likely that the altered phosphorylation and temperature responses are a result of an abnormal lipid environment of either the enzymes or their substrates.

John Vickers is a Post-Doctoral Fellow of the Muscular Dystrophy Association of Canada. This is supported by a group grant from the Medical Research Council of Canada, MRC Group in Developmental Neurobiology.

706 ULTRASTRUCTURAL STUDIES OF MEMBRANE CHANGES DURING NEURONAL AGGREGATION. John G. Wood, Barbara J. McLaughlin, and Frances I. Byrd*. Dept. Anat., Univ. Tenn. Center for the Health Sciences, Memphis, TN 38163.

The use of reaggregating nervous system cultures provides a useful model for the study of many aspects of cellular associations during development of the nervous system but as yet there has been little use of electron microscopy to study this problem. Our approach is to utilize immunocytochemical methods to study the changes in membrane associated components on aggregating cells and, in order to obtain baseline information on changes in normal membrane morphology during reaggregation, cell or aggregate suspensions taken between 0 hr. to 14 days after dissociation are prepared for either standard thin section or freeze fracture analysis. The cells are rounded and dispersed freely at 0 hr. after dissociation. Between 2 and 6 hr. the cells become closely apposed on all sides by other cells and form small aggregates. At this time puncta adhaerentia junctions and focal gap and tight junctions are present between apposed cell bodies and processes. Between 1 and 5 days, more growth cones and neurites are present and fewer gap and tight junctions are seen. Synapses form between 5 and 7 days and continue to increase in number up to the fourteenth day. By 9 days, the peripheral border of each aggregate is composed of cells having numerous microvilli on their free surfaces and which are joined by zonula adhaerens junctions. Freeze fracture analysis of the early reaggregating cells (2-4 hr.) reveals a sparse intramembrane particle distribution on the cytoplasmic and external membrane leaflets of apposed cells. By 2 days intramembrane particles on the cytoplasmic leaflet of apposed cells are more numerous and small clusters of loosely packed particles are seen, which may represent junctional contacts between reaggregating cells. These particle clusters increase in number and size as reaggregation proceeds. By 7 days other particle arrays which correspond to intramembrane specializations characteristic of excitatory synaptic junctions are seen on cell bodies and neurites with increasing frequency. These observations suggest that correlated cell surface and intramembrane changes are occurring during the initial stages of neuronal reaggregation, which may reflect a basic morphological reorganization of cell membranes during neuronal recognition and synaptic junction formation. Such changes may be studied by cytochemical analysis utilizing ligand specific probes or antibodies to membrane components together with labeling techniques for electron microscopic localization.
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707 CALCIUM TRANSPORT ACROSS BLACK LIPID MEMBRANES (BLM) AS DETERMINED WITH AEQUORIN. Miko Yoshida*, Arthur F. Clark*, W. L. Stahl* and P. D. Swanson. Dept. of Med. (Neurology), Biophy. and Ped., Univ. of Wa. Sch. of Med., Seattle, Wa. 98195.

Transport of calcium across an oxidized cholesterol bilayer membrane was monitored by following the change in the rate of light emission due to the reaction of the transported calcium with aequorin, a calcium sensitive protein. We used a semispherical bilayer bubble with aequorin present within the bubble, and a buffered external calcium solution. The semispherical membranes were formed using an apparatus similar to that described by Toyoshima and Thompson (Bioch. 14, 1518, 1975) and were suspended in a spectrophotometric cell. Electrodes, Ag/AgCl, were used to measure transmembrane potential and membrane conductance. The design of the apparatus allowed for exchange of both the internal and external solutions. A photomultiplier tube was positioned close to the spectrophotometric cell and the output was monitored on an oscilloscope and recorded. When the external solution contained 100mM KCl, 8mM Tris and 1mM CaCl and the internal solution contained the same solution but without calcium and containing aequorin, the membrane conductance was 5.5×10^5 ohm x cm^2 and the rate of light emission low. Upon addition of the calcium ionophore X-537A the membrane conductance increased in a time dependent manner and there was a concomitant increase in the rate of light emission. Quantification of the light changes were complicated by an increase in both the size and frequency of the pulses. In a second series of experiments we prepared two concentric bubbles with aequorin present only within the innermost bubble. This was done in order to isolate the aequorin so that it could not interact with the external membrane of interest. Thus electrical and physiochemical changes due to the addition of possible calcium transport protein or ionophores would not be affected by the presence of aequorin. Using the conditions above with KCl, Tris and CaCl in the external solution and the aequorin isolated within the inner bubble the conductance of the external membrane was 5.5×10^7 ohm x cm^2. As in the first system X-537A caused both an increase in the membrane conductance and the rate of light emission.
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708 INTERNAL PERFUSION TECHNIQUE IN THE TUNICATE EGG AND EFFECTS OF INTERNAL IONIZED Ca UPON EGG Na and Ca CHANNELS. Mitsunobu Yoshii* and Kunitaro Takahashi* (SPON: M. Maeda). Inst. Brain Research, Univ. Tokyo Med. Sch., Tokyo, Japan.

Previous studies on the development of the excitability in the embryonic membrane of the tunicate have shown that the unfertilized egg membrane is excitable, having Na, Ca, delayed K, and anomalous K channels. In the differentiated tadpole larva, Ca channels were segregated in the muscle membrane, while Na channels were accumulated in ectodermal cells. During the development the assembly of ionic channels on the embryonic membrane must be modified by factors inside the egg cell. In the present study the internal perfusion technique was developed to analyse the effect of internal Ca ions upon Na and Ca channels in the tunicate egg membrane.

The perfusion apparatus consisted of the upper and lower compartments which were connected by a small funnel made from a piece of Pyrex capillary. The egg cell without follicular envelope was dropped in the funnel and brought into contact with the glass wall. The completion of the contact was checked by an increased resistance of more than 1000 Mohms between the compartments. Then,the lower part of the free membrane was ruptured by slight negative pressure or injection of high frequency current. Internal solution which contained 400 mM Na, F or Cl as anions, and 100 mM EGTA to regulate ionized Ca, was perfused in the lower compartment. The upper compartment was filled by external solution containing 400 mM NaCl and 100 mM $CaCl_2$ or $SrCl_2$. After the rupture the upper membrane was voltage-clamped and the holding potential was set at -90 mV at the internal side. Step potential changes revealed the same Na current as observed in the intact egg. The efficiency of the perfusion of the internal solution was confirmed by the negative shift of the reversal potential of Na channel current. The reversal potential became less than 5 mV with 400 mM internal Na 20 min after the rupture and increased beyond 85 mV after the replacement of 400 mM Na with 400 mM Cs. Therefore the efficiency of the exchange was more than 95% in 30 min with the present method. By using F ions as internal anions the preparation was kept stable more than two hours and large Na but no Ca currents were observed. By using Cl ions, however, both Na and Ca currents were observed as in the intact egg, although the stable condition could not be kept more than 30 min. An increase in ionized Ca from 10^{-7} to 8×10^{-4}M in the external solution containing Cl ions caused a reduction of Ca current by one fifth and enhanced Na channel conductance about twice. The reciprocal effects of internal Ca ions upon the egg Na and Ca channels suggested that the internal Ca ions have an important role in changing channel density of the embryonic membrane during development and differentiation.

709 ISOLATION AND CHARACTERIZATION OF NORMAL HUMAN CNS AXOLEMMA-ENRICHED FRACTIONS. W.J. Zetusky*, V.P. Calabrese, G. Anderson*, and G.H. De Vries. Depts. of Biochemistry and Neurology, Med. Coll. of Va. and McGuire VA Hospital, Richmond, VA 23298

A 3% homogenate of corpus collosum from frozen human brain was prepared in 0.85 M sucrose, .15 M NaCl, 0.01 M TES, pH 7.5 (Medium A) using 10 strokes of a Dounce homogenizer. Centrifugation of this homogenate (82,500 xg for 15 min) yielded a floating layer of myelinated axons plus free myelin and a pellet of debris, myelin-free axons and capillaries. The myelinated axon pad was further purified by rehomogenization and reflotation two times in Medium A. The myelinated axons were osmotically shocked in 10 mM TES, pH 7.5 and centrifuged at 82,500 xg for 30 min to form a pellet. The pellet derived from 1 gm of starting white matter was resuspended in 0.65 M sucrose containing 1 mM EDTA and 1 mM TES, pH 7.5 and was layered on a 3 step discontinuous gradient of 1.2 M, 1.0 M and 0.8 M sucrose. All sucrose gradient solutions contained 1 mM EGTA and 1 mM TES, pH 7.5. After centrifugation at 82,500 xg for 1 hour, 4 fractions were obtained: myelin floating in the 0.65 M sucrose layer, two axolemma-enriched fractions sedimenting to the 0.8 M/1.0 M and 1.0 M/1.2 M sucrose interfaces, and a pellet of myelin-free axons. The yield of 0.8/1.0 and 1.0/1.2 was 0.13 mg and 0.40 mg protein respectively per gram wet weight of starting white matter. The enrichment in specific activity over the starting whole white matter homogenate was two to four fold for 5' nucleotidase, approximately two fold for acetylcholinesterase and 6 to 16 fold for (Na^+-K^+) activated ATPase. The specific activity of 2'3' cyclic nucleotide 3' phosphohydrolase was slightly lower than whole homogenate in the 1.0/1.2 fraction but slightly higher in the 0.8/1.0 fraction. The specific activity of cytochrome c oxidase, antimycin resistant NADH oxidase, and antimycin sensitive NADH oxidase showed that the fractions were not significantly contaminated with mitochondrial inner membrane, microsomes or mitochondrial outer membrane. Specific radioimmunometric assays showed that less than 4% myelin basic protein was present in the 1.0/1.2 fraction and that less than 0.2% glial fibrillary acidic protein was present in either axolemma-enriched fraction. The 0.8/1.0 fraction contained predominantly unilamellar membrane plus some typical multilamellar myelin fragment vesicles. The 1.0/1.2 fraction contained mostly unlamellar membrane vesicles with only occasional myelin fragments. The data suggests that the majority of the unilamellar membranes in the 0.8/1.0 and 1.0/1.2 fractions are derived from the axonal plasma membrane.
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MEMORY AND LEARNING

710 THE EFFECTS OF IMMEDIATE POST-TRAINING LOCUS COERULEUS DAMAGE ON THE LONG TERM PERSISTENCE OF MEMORY. W.C. ABRAHAM,*Ge Ruigt* and S.F. Zornetzer. Dept. Neuroscience, Sch. Med. Univ. Fla. Gainesville, Fl., 32610.

One of the least studied and most poorly understood of these component processes is the long term persistence of memory. This study investigated the role of the norepinephrine (NE) containing locus coeruleus (LC) in the persistence of memory. Previous work from our laboratory (Zornetzer & Gold, 1976; Zornetzer & Appleton, 1977) indicates that electrolytic lesions of the LC made immediately after mice learn a single trial inhibitory avoidance response, results in a prolonged period (at least 196 hours) of memory susceptibility to retrograde disruption by ECS. This prolonged period of susceptibility suggests that "consolidation" into a long term stable state is delayed following LC damage. Is the duration of the long-term stable memory increased by a period of time equivalent to the extended duration of the labile state? Or, is the long-term stable trace initiated at the time of training and unaltered by persistence of the labile trace?

Nichrome wire electrodes were stereotaxically implanted in the region of the LC bilaterally in male Swiss mice. Seven days after surgery all mice were trained in a single trial inhibitory avoidance apparatus. Immediately following training mice were lightly etherized and electrolytic destruction of the LC was made (anodal current, 300ua for 10"). Mice were returned to their home cages and tested for retention of the inhibitory avoidance response either 4, 6, 7, or 8 weeks after training. Forgetting curves were obtained for all mice. LC lesioned mice showed long-term persistence of memory identical to operated controls and normal mice. Thus, all groups had excellent retention at 6 weeks with an intermediate level at 7 weeks and virtually complete forgetting by 8 weeks.

These data indicate that forebrain levels of NE, normally provided by LC neurons, are not necessary for the persistence of memory over long periods. Further, since LC lesions result in a prolonged labile memory state these data also indicate that the initiation of the long-term stable memory is unaltered by post-training LC destruction since its persistence is identical in lesioned and control mice. These data suggest that two parallel states of memory exist. The first, a labile trace, ordinarily called STM, has a duration normally delimited by forebrain NE. The second, a long lasting stabile trace, ordinarily called LTM, is independent of NE biosynthesis by the LC, and therefore unaltered by immediate post-training LC destruction. The interactions of these two memory states and the resulting implications for traditional consolidation theory will be discussed.

711 Escape Deficits Following Exposure To Inescapable Shock: Dopaminergic And Noradrenergic Involvement. Hymie Anisman* and Lawrence S. Sklar* (SPON: W. G. Webster). Department of Psychology, Carleton University, Ottawa, Ontario, Canada.

Following exposure to inescapable/unavoidable shock subsequent escape performance is severely retarded. The performance disruption appears to reflect deficits in response initiation and maintenance rather than cognitive changes (helplessness). Specifically, the performance deficits occur only when escape responding is briefly prevented during test. This treatment does not affect performance of mice not previously exposed to inescapable shock. Treatment with catecholaminergic agents mimicked the effects of inescapable shock. Treatment with alpha-methyl-para-tyrosine (AMPT) (125 & 250 mg/kg), Bis-(4-methyl-1-homopiperazinyl-thiocarbonyl) disulfide (FLA-63) (40 & 60 mg/kg), haloperidol (.075, .15, .30, .45, & .60 mg/kg) or pimozide (.40 & .80 mg/kg) produced dose dependent escape deficits. With increasing dosages the magnitude of the deficit was accentuated, and shorter escape delays successfully elicited the performance disruption. As in the case of inescapable shock, prior escape training immunized against the disruptive influence of the drug treatments. Finally, low dosages of DA or NE synthesis inhibitors or receptor blockers, together with small numbers of inescapable shocks produced performance deficits beyond that elicited by either treatment alone. Scopolamine (.5 & 2.0 mg/kg) antagonized the effects of both inescapable shock and haloperidol. Predictably L-3,4-Dihydroxyphenylalanine methyl ester (L-DOPA) antagonized the effects of inescapable shock. It is concluded that both DA and NE, as well as ACh, mediate the escape deficits via their influence on response initiation and maintenance.

712 ERGODICITY IN PROBABILISTIC NEURAL NETS. Photios Anninos, Silvio Zenone*, Max Papadopoulos*. Dept. of Phys. Concordia University and Phys. Dept. Dawson College, Montreal, P.Q.

In a series of articles, Harth et al (1) and Anninos et al (2) have developed the dynamics of probabilistic neural nets. Harth and Edgar (3) have also shown how a completely random net can become structured as a result of sensory inputs and can, as a result, be made to perform a variety of learned tasks.

The idea that use leads to structuring in the CNS is the fundamental to the work of many workers. Eccles, in a series of articles and papers makes the case that "learning behaviour signals changes that have occurred in neuronal connectivity within the CNS". Eccles's explanation is that the basic mechanism of this process is synaptic facilitation. (4)

Changes in the connectivity pattern of probabilistic neural nets can be studied with the Anninos model. In fact hysteresis effects have been observed in the study of netlet activity. These changes in netlet activity are the result of variation in the connectivity parameters of the system. (5)

The purpose of current work is to pursue the latter idea further and to investigate hysteresis behaviour in its correlation to various learning paradigms. Information Theory and Entropy concepts are utilized in this regard.

An Entropy model suitable for probabilistic neural nets has been developed by Bergstrom (6) and by Bergstrom and Nevanlinna. (7). The results of Bergstrom and Nevanlinna are being utilized for the purpose of associating a suitable function to the hysteresis graph.

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713 THE EFFECTS OF DELAYED SCOPOLAMINE ADMINISTRATION UPON MEMORY FOLLOWING LOCUS COERULEUS LESIONS. Robert S. Appleton*, A.J. Dunn and S.F. Zornetzer. Dept. Neuroscience, Coll. Med., Univ. Fla., Gainesville, Fla., 32610.

In previous studies we reported that posttraining electrolytic lesions of the locus coeruleus (LC) complex results in a long-lasting period of memory susceptibility to ECS produced retrograde amnesia (Zornetzer & Appleton, 1977). Memory retrieval during this prolonged period of susceptibility is, however, normal. Since the LC-mediated norepinephrine (NE) neurotransmitter system is deficient in these mice, the question arises, to what extent are other neurotransmitter systems involved in the maintenance of this long-lasting labile trace? Based upon the suggestions of a number of previously published papers, we chose to investigate initially the possible role of the cholinergic system in the maintenance of this labile memory trace.

Male Swiss ICR mice were prepared with chronic twisted wire electrodes targeted for the LC bilaterally. Seven days after surgery mice were trained on the single-trial inhibitory avoidance step-through apparatus. Immediately following training mice were lightly etherized and anodal lesions were made bilaterally (300 ua, 10"). Mice were returned to their home cages for 24 hr, at which time they were given an injection of either scopolamine HBr (1.0 or 2.0 mg/kg) or saline I.P. Following injection micewere returned to their home cages. Twenty four hrs after injection all mice were tested for retention of the inhibitory avoidance response. Following testing histological analysis of electrode placements was performed.

The results suggest that LC lesioned mice injected with scopolamine 24 hrs after training are impaired in their subsequent avoidance behavior. The data are discussed in terms of multiple and redundant neurotransmitter systems and memory.

714 THE EFFECTS OF AGING ON PRIMATE SHORT-TERM MEMORY, SENSORY PROCESSING AND LEARNING. Raymond T. Bartus, Denise Fleming* and H. R. Johnson*. CNS Pharmacology, Warner-Lambert/Parke-Davis Pharmaceutical Res. Div., Ann Arbor, Mich. 48106

The performance of five aged rhesus monkeys (17 years and older) was compared to that of five young monkeys (three to five years old) in a series of experiments designed to define the cognitive impairments associated with aging. An automated test apparatus (specifically designed to control or eliminate potentially confounding variables involving general attention, motivation, psychomotor coordination, etc.) was used for all experiments.

In the first experiment, short-term memory (STM) was assessed using an indirect delayed response procedure. It was found that aged monkeys suffer a severe STM deficit, performing quite accurately under control conditions, where little or no STM was required, but exhibiting marked impairment of performance after retention intervals of 15 and 30 sec.

The second and third experiments evaluated the extent to which impairments in sensory processing might be responsible for the age-related STM impairment. Sensory processing ability was evaluated by manipulating either the number of times the stimulus was flashed, or the duration of a single stimulus flash before the delayed-response retention interval was initiated. The results demonstrated that, although performance generally suffered when the stimulus information was reduced in this manner, no differential age-related effects in sensory processing occurred.

In the final experiment, learning ability was assessed using a two-choice visual discrimination paradigm. Color, pattern and pattern reversal discrimination problems were administered. Acquisition rates revealed no consistent discrimination learning impairments in aged monkeys, even though some discrimination problems were clearly more difficult to learn for both age groups. However, a marked reversal learning impairment was observed in the aged group. Thus, although original learning may not be adversely affected by age, the ability to modify previously learned habits apparently becomes quite impaired.

These data not only share striking similarities to much of the human geriatric literature, but are also in excellent agreement with data from frontally ablated monkeys. It is therefore suggested that the aged monkey provides an excellent neurobehavioral model to study impairments and processes associated with aging, and that a major neurological cause of the impairments may involve a dysfunction in frontal cortical and related anatomical areas.

715 ENKEPHALIN- AND MORPHINE-INDUCED FACILITATION OF LONG-TERM MEMORY. James D. Belluzzi and Larry Stein. Wyeth Laboratories, Philadelphia, Pa. 19101.

A role for enkephalin in the mediation of behavioral reinforcement has been suggested by several lines of evidence: (1) central injections of enkephalin may serve as reinforcement for self-administration behavior, (2) electrical stimulation of many enkephalin-rich regions, such as midbrain central gray, may support high rates of self-stimulation, and (3) such brain stimulation reinforcement may be blocked by low doses (0.5-2.5 mg/kg, s.c.) of naloxone (Belluzzi and Stein, 1977). The important role of reinforcement in learning and long-term memory formation, although controversial at the level of theory, cannot be disputed at the practical level. Accordingly, enkephalin and morphine treatments were administered immediately following training in a single-trial learning task in an attempt to facilitate long-term retention of the learned response. Rats with permanently-indwelling intraventricular cannulae received a mildly painful foot shock after stepping down from a shelf to a grid floor. Different groups received intraventricular injections either immediately or 15 minutes after the shock, as indicated in the Table. Three days later, long-term memory of the shock (as reflected by long step-down times) was measured in a retention test. Significant facilitation of the learned response was observed in the groups given immediate post-shock treatments of methionine-enkephalin (200 µg) or morphine. Two further observations suggest that these effects of the opiates on retention were due to an enhanced memory of the shock. First, the morphine treatments were ineffective if the shock was withheld on the training day. Second, delaying the morphine injection for only 15 minutes after the shock completely abolished its memory-enhancing effect. These results suggest that post-trial activation of opiate (enkephalin) receptors may facilitate memory consolidation.

Treatment	Dose (µg)	No. of Rats	Step-Down Time (Sec.) Mean ± S.E.M.
Ringer's	-	17	36.23 ± 9.37
Morphine	20	19	78.24 ± 12.14*
Morphine (Shock withheld)	20	8	5.75 ± 0.94**
Morphine (15 min. delay)	20	7	39.50 ± 23.70
Leu-Enkephalin	100	10	49.18 ± 22.02
	200	8	47.04 ± 19.71
Met-Enkephalin	100	5	8.82 ± 1.81
	200	10	114.25 ± 27.74*

* $p<.02$ vs. Ringer's; ** $p<.001$ vs. Morphine

716 HIPPOCAMPAL EEG PREDICTORS OF LEARNING RATE IN RABBITS. Stephen D. Berry* and Richard F. Thompson. (SPON: S.R. Coates). Dept. Psychobiology, UCI, Irvine, CA. 92717.

Spontaneous EEG samples were recorded from the CA1 area of dorsal hippocampus in 17 New Zealand White rabbits (Oryctolagus cuniculus) prior to the beginning of classical conditioning of the nictitating membrane (NM) reflex. The conditioning paradigm consisted of a tone CS (350 msec, 85 db, 1 KHz) and corneal air puff US (3 psi, 100 msec, overlapping and coterminating with the CS). A conditioned response (CR) was defined as any NM movement greater than 0.5 mm occurring after CS onset but before US onset in paired trials or within 500 msec of CS onset in probe (CS alone) trials. Rabbits were conditioned until they reached a criterion of 8 out of 9 consecutive CRs or for a maximum of 468 trials (4 sessions).

The 2 min preconditioning EEG samples were analyzed on a PDP-12 computer by a zero crossing program which determined the period between consecutive baseline crossings in the EEG, and accumulated the number of waves occurring in frequency categories (0-14 Hz in 2 Hz increments; 14-22 Hz in 4 Hz increments). Pearson Product-Moment coefficients were computed between the number of trials needed to reach criterion and the amount of time (number of waves times the period of the mean frequency of that category) in each of the frequency categories. In general, time scores in the theta (2-8 Hz) range were negatively correlated with trials to criterion (slower learning), while the higher frequency categories (8-22 Hz) showed positive correlations. The strongest overall relationship was between learning trials and waves in the 8-10 Hz range ($r=+.72$, $df=15$, $p<.01$). It was concluded that preconditioning hippocampal EEG is a reliable predictor of subsequent rate of NM conditioning in rabbits.

717 SHORT TERM MEMORY IN THE RAT: EFFECTS OF ELECTRICAL STIMULATION OF MIDBRAIN RETICULAR FORMATION. A. Rex Bierley* and Raymond P. Kesner. Dept. Psychol., Univ. of Utah, Salt Lake City, UT 84112.

An attempt was made to delineate the role of the midbrain reticular formation (MRF) in the modulation of short term memory (STM) processes. Long-Evans rats with bipolar electrodes implanted in the MRF were trained on a discrete-trial delayed alternation task. The task required the rat, in a two bar choice situation, to press the bar opposite to the one he pressed in initiating the trial in order to obtain reinforcement. Time between initiation of a trial and presentation of the two bars was varied from zero to 60 seconds. Rats trained on this task show a retention function (as inferred from performance) that decays within 60 seconds, reflecting a time course interpreted as a STM gradient. Low intensity electrical stimulation (40-70µA, 100Hz., 0.1msec pulse width) of brief duration (5 seconds) delivered to the MRF contingent upon an animal initiating a trial produced chance responding at all retention intervals. In a second experiment, the locus of this effect was studied by measuring performance at a 15 second retention interval and by varying the temporal presentation of MRF stimulation. It was shown that MRF stimulation delivered immediately, 5, or 10 seconds after the start of the trial produced chance responding. The same stimulation delivered prior to the start of a trial had no effect. Furthermore, no within-sessions proactive interference effects were observed. In additional experiments, it was demonstrated that disruptive effects were not due to interference with a motor set or to rewarding or punishing consequences of the stimulation.

The data suggest that the MRF plays a critical role in processes associated with persistence of information within a STM system.

718 EFFECTS OF BILATERAL AUDITORY CORTEX ABLATION ON BEHAVIOR AND UNIT ACTIVITY IN RAT INFERIOR COLLICULUS DURING DIFFERENTIAL CONDITIONING. Dorwin Birt*, Robert Nienhuis*, and James Olds (SPON: Frank Atencio)
California Institute of Technology, Pasadena, Calif. 91125

The development of both behavioral responses and short latency responses of inferior colliculus neurons to tone pips was studied in normal rats and rats with bilateral ablation of auditory cortex during a differential conditioning experiment. Behavior measured was the development of a differential movement during the one second interval between the onset of the tone CS+ and the food pellet US. Some animals with small lesions learned this discrimination but no animal with large lesions did so. Unit responses during the period 5-11 msec after stimulus onset were measured before and during conditioning. No apparent relationship was found between changes in these short latency responses and the pairing of the CS+ with food pellets in normals or lesioned animals. There was, however, a large effect of the lesions on the variability of the background firing rates of neurons during conditioning but not during pseudoconditioning. In normal animals an approximately equal number of units showed background increases and decreases during conditioning. In the lesioned animals there were more background increases and almost no decreases during conditioning. This was interpreted as favoring the idea that during the learning of such a discrimination a non specific activation from non cortical structures is selectively antagonized by cortical efferents.

719 DOES REWARDING HIPPOCAMPAL STIMULATION DISRUPT FOOD-REINFORCED LEARNING IN RATS? Mauro Caudarella,* K. A. Campbell* and N.W. Milgram, Dept. Psychol., Scarborough Coll., Univ. Toronto, West Hill, Ont., Canada, M1C 1A4.

Electrical stimulation of the dorsolateral hippocampus (HPC) has been used, in separate experiments, both to produce retrograde amnesia for recently-learned behaviors, and to reinforce operant behavior in self-stimulation experiments. These apparently contradictory effects led us to determine whether HPC stimulation would disrupt appetitive learning when stimulation follows each lever-press as in self-stimulation experiments. 23 hooded rats, stereotaxically-implanted with single monopolar electrodes in the dorsolateral HPC (CA3), were maintained at 85% of their body weight and randomly divided into 3 groups tested in identical Skinner boxes: one group received both a food pellet and .5 sec of 30μA, 60 Hz, sine-wave current immediately following each lever-press ("contingently-stimulated" group); a second group received a food pellet for each lever-press as well as .5 sec of stimulation whenever a paired animal in the contingently-stimulated group depressed the lever ("yoked control" group); and a third group received only food pellets, without stimulation ("surgical control" group). After familiarization with the food dispenser, the rats were left unassisted to learn to press the lever. Lever-presses were recorded every 15 min during daily 30-min sessions. The 2 contingently-stimulated rats which had not learned in 7 days were "shaped" successfully by the method of successive approximations. After 750 lever-presses, the rats were screened for self-stimulation and the data from only those rats that self-stimulated within 9 daily sessions were analyzed. The Kruskal-Wallis test indicated significant differences in acquisition ($p<.05$). The contingently-stimulated group required more trials than either control group to reach both an initial acquisition criterion of 35 lever-presses in 15 min and a more conservative learning criterion of 550 cumulative lever-presses. Thus food-reinforced learning was disrupted by HPC stimulation contingent on each lever-press, in spite of the fact that all animals in this group eventually self-stimulated. HPC stimulation delivered in more random fashion (yoked control group), however, did not disrupt learning. Surprisingly, when transferred to an identical Skinner box without a food pellet dispenser, contingently-stimulated animals and yoked controls were both slow to self-stimulate, requiring a mean of 5 daily sessions to acquire lever-pressing for HPC stimulation. The lack of immediate transfer in the contingently-stimulated group may indicate that the effects of HPC stimulation vary over time and such variation may account for the reported differential amnestic and rewarding effects of HPC stimulation.

720 RECOVERY OF MEMORY AS A FUNCTION OF THE DEGREE OF AMNESIA DUE TO PROTEIN SYNTHESIS INHIBITION. Hasker P. Davis, Mark R. Rosenzweig, Edward L. Bennett and Anne E. Orme* Psychology Dept., and Lawrence Berkeley Lab, Berkeley, CA 94720, USA.

Retrograde amnesia following inhibition of cerebral protein synthesis has generally been explained as being due to either a failure of consolidation or impairment of a retrieval mechanism. Major evidence claimed to support the retrieval hypothesis is provided by studies which utilize a reminder (usually footshock) to attenuate the amnesic consequence of the protein inhibitor. To examine this question, male Swiss Webster CD-1 mice were injected subcutaneously with anisomycin (1 mg/animal, 7 mg/animal, or 1 mg/animal every 2 hr x 7) and given one training trial in a passive avoidance box. All 424 subjects received a single retention test on each of four consecutive days, starting either 1, 7, or 21 days after training. One-half of the mice in each group received a footshock reminder 1 hr after their initial test. The degree of amnesia was significantly greater at longer training-test intervals and in groups treated with a high (7mg/animal) or multiple (1 mg every 2 hr x 7) dosage of anisomycin. The footshock reminder did not attenuate the inhibitor-induced amnesia ($p< 0.25$). Multiple testing did produce partial recovery but only in those animals that demonstrated some memory of training as indicated by their initial step-through latencies (STL). Most importantly, a comparison of STLs of saline animals first tested at 21 days with the STLs of anisomycin treated animals (1mg/animal) first tested at 7 days showed that these groups were similar in their initial poor retention and pattern of recovery on test days 1-4 ($p>0.30$). Groups that demonstrated complete amnesia on their initial test showed no recovery on subsequent tests. A within-group analysis of performance, irrespective of drug or training test interval, demonstrated that the degree of retention, as indicated by the initial STL, was the primary indicator of whether or not an animal would show recovery. These results indicate that recovery is to be expected in partially amnesic animals but not in animals completely amnesic; thus they provide strong support for the consolidation hypothesis.

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721 RETROGRADE AMNESIA IN CHICKS: INDUCTION BY L-BAIKIAIN IS NOT ACCOMPANIED BY EEG SEIZURES OR DEPRESSION. Joel L. Davis, Lauren K. Gerbrandt*, Arthur Cherkin, Psychobiology Research Laboratory, VA Hospital, Sepulveda, CA 91343 and Dept. Psychology, California State University, Northridge, CA 91324.

Intraventricular injection of L-proline (L-PRO) induces retrograde amnesia without causing brain seizure or isoelectric activity, whereas D-PRO is non-amnesic. We now report that the proline analog L-baikiain (L-BAI; 4,5-dehydro-L-pipecolic acid) has similar effects at a lower dose. We injected chicks intracerebrally with 5 μl/hemosphere of L-BAI, L-PRO or D-PRO (150 mM, pH 7.2 ± 0.2), 1 min after one-trial training to suppress the spontaneous peck response to a 3-mm stainless steel bead. Avoidance of the attractive bead was conditioned by coating it with an aversive liquid (methyl anthranilate) immediately prior to training. Retention of the avoidance response was tested 24 hr later using the uncoated bead; reduced avoidance scores and increased peck scores indicate impaired memory retention.

Compound	Dose/Chick (μmols)	N	Avoidance Score (%)	Peck Score ($\sqrt{p}$ ± S.D.)
L-Baikiain	1.5	59	30.5	1.51 ± 1.23
L-Proline	1.5	59	49.2	0.85 ± 1.05
D-Proline	1.5	60	61.7	0.78 ± 1.10
L-Proline	6.0	304	34.5	1.59 ± 1.49
D-Proline	6.0	296	56.1	0.77 ± 1.15

The results demonstrate that L-BAI is an effective retroactive amnesic agent compared to D-PRO controls; the avoidance scores ($p<0.002$; χ^2 test) and peck scores ($p<0.001$; t-test) are significantly different. The amnesic effect induced by 1.5 μmols of L-BAI is comparable to that found with 6.0 μmols of L-PRO in previous experiments (see table). Higher doses of L-BAI (3.0-6.0 μmols) had lethal effects ($LD_{50} \approx 4.5$ μmols) not seen with L-PRO ($LD_3 > 12$ μmols).

We also examined the electrophysiological effects produced by L-BAI. Chicks were chronically implanted with active bilateral recording electrodes in the ectostriatum, an indifferent electrode under the comb, and a ground electrode in dorsal neck muscle. The following day, after a 15-min adaptation and EEG baseline period, chicks (N=6/group) received intracerebral injections of 1.5 μmols of L-BAI or D-PRO. Multiple-unit activity and raw integrated EEG activity were recorded for 10 min after injection. No seizure spiking or isoelectric activity was observed in polygraph records. No reliable differences in EEG and MUA reaction were observed after L-BAI versus D-PRO injections.

We conclude that L-BAI is approximately 4x more potent an amnesic agent than L-PRO; both are effective at non-lethal doses which cause no marked electrophysiological changes.

722 DEVELOPMENT AND TOPOGRAPHY OF CLASSICALLY CONDITIONED FLEXION REFLEX FACILITATION IN SPINAL CAT. Russell G. Durkovic. Dept. Physiol., Upstate Med. Ctr., Syracuse, N.Y. 13210 USA.

In previous analyses of classically conditioned flexion reflex facilitation in spinal cat preparations only parts of the conditioned response data have been used to measure changes in reflex activity. For example, Patterson et al. (JCPP 84:88, 1973) measured only the response to the first pulse of the conditioned stimulus (CS); Durkovic (Physiol. and Behav. 14:297, 1975) measured only the maximum tension observed during each CS presentation. In the present study the data from 30 conditioning and 30 sensitization control animals were analyzed over the entire duration of the CS. Of primary interest was first to see how rapidly the conditioned facilitation developed, and second to examine the topography of the facilitation over the course of each CS presentation.

In unanesthetized, decerebrate cats the spinal cord was transected at T-10. The tendon of the tibialis anterior muscle of the rigidly fixed hind limb was attached to a force-displacement transducer. Electrical stimulation of the cutaneous saphenous nerve of this leg served as the CS (10 i.p.s. for 1.5 secs). This train of stimuli was repeated once each minute for both conditioning and sensitization control animals. Each pulse of the CS evoked a reflex response in the muscle. The peak tension of each response was measured during CS alone trials, in acquisition and in extinction procedures for each animal. On the 30 acquisition trials, conditioning animals received electrical stimulation of the cutaneous superficial peroneal nerve at 30 ips (the unconditioned stimulus (US)) during the last 0.5 seconds of each CS presentation. In sensitization control animals the CS and US presentations were alternated every 30 secs. On extinction trials all animals received CS-only presentations.

By acquisition trial 2, consistently significant differences between conditioning and sensitization animals developed for the 3rd and 4th CS peaks (200-300 msec after CS onset). By acquisition trials 5-7 a consistent difference developed between conditioning and sensitization animals for each of the 10 peaks in the first second of each CS presentation. Response topography for both conditioning and sensitization control animals on these trials was essentially flat. However, during extinction trials some conditioned animals appeared to exhibit behavior reminiscent of the POST-US-R response pattern observed in human GSR conditioning studies, e.g., Lockhart (Psychophysiology 10:112, 1973).

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723 BLOCKADE AND ACTIVATION OF CAUDATE CHOLINERGIC ACTIVITY. EFFECTS ON PASSIVE AVOIDANCE. Maribel Fernández Samblancat*, Marcos Solodkin Horowitz* and Roberto A. Prado-Alcalá. (SPON: Juan A. Roig). Dept. Psychophysiol. Sch. Psychol. Anáhuac Univ. and Physiol. Dept. Med. Sch. Ntnl. Univ. of México, México City, México.

It has been shown that cholinergic blockade of the caudate nucleus (CN) interferes with performance of positively reinforced behaviors as well as with active avoidance. In this paper we present evidence that passive avoidance (PA) is also mediated by a caudate-cholinergic mechanism. PA was studied in rats (Ss) using a two-compartment box. Ten sec after being put into the safety compartment, Ss were allowed to step into the gridded compartment where all received a footshock. Their latency to step into the latter compartment was measured, again, 24 (Group I) or 48 (Group II) hr later (retention test).

Different subgroups within Group I were studied: unimplanted, implanted with cannulae in the CN, and implanted in the parietal cortex. Microinjections of saline or of 3 different doses of atropine were tested. Group II had 3 subgroups: unimplanted, microinjected in the CN, with saline, or microinjected with choline. There was one treatment per group and all microinjections were bilateral, performed 6 min after the first (footshock) session.

When retention of PA was tested 24 hr after session one (Group I) a dose-dependent deficit was found in the caudate subgroups treated with atropine, whereas a much smaller deficit was found in the cortical atropine-injected subgroup. In Group II retention scores, from least to most were obtained by the saline $<$ unimplanted $<$ choline subgroups, the latter showing an increase in retention latency of more than 300% as compared with the unimplanted subgroup.

These results suggest that PA is also dependent on a caudate-cholinergic mechanism.

724 ULTRASTRUCTURAL CHANGES IN THE DENTATE MOLECULAR LAYER DURING CONDITIONING. Eva Fifkova, Barbara J. Van der Wege* and A. Van Harreveld. Dept. Psych., Univ. Colo., Boulder, CO 80309 and Calif. Inst. Technol., Pasadena, CA 91125

A long-lasting increase in the area of dendritic spines was observed in the dentate molecular layer following a single tetanic stimulus to the perforant path (Fifkova, Van Harreveld: J. Neurocyt. 6:211,1977). Likewise tetanic stimulation of the perforant path yields long-lasting postactivation potentiation (Bliss, Lømo: J. Physiol. 232:331,1973; Douglass, Goddard: Brain Res. 86:205, 1975) which suggests a causal relation between the two phenomena. Since increased unit activity in the dentate was observed in classical conditioning paradigms (Segal: Thesis, 1972; Thompson: Amer. Psych. 31:209,1976), present experiments were aimed at a search for morphological changes in the dentate molecular layer during conditioning. Forty-four mice (20g) had twice a day 50 pellets (45mg) available for 2 consecutive days in cages where the conditioning was to take place. On the third day fifty trials of tone followed by a pellet were presented in a single 2-hr session. Controls spent comparable time in the training cage with 50 pellets available. Following 90 min mice were sacrificed and prepared for electron microscopy. The area of dendritic spines in the dentate molecular layer was significantly larger in the middle and distal third of conditioned mice as compared to controls. Since fewer pellets were consumed by the conditioned animals than by controls, the former were divided into 3 groups relative to the pellet consumption (>75%; 50-75%; <50%). An inverse relation between these values and the magnitude of spine enlargement (Table 1), in spite of similar starting weights and weight loss, could point to unspecific factors, like stress, to be partly involved in the observed spine change. The increment in pellet consumption between the last feeding and during conditioning in group <50% was significantly lower. Since spine change was here the largest one, it could indicate that the entire procedure was stressful. However these animals ate at a lower rate throughout, so that the magnitude of spine change might also indicate that this group performed at its maximum. The increment in groups >75% and 50-75% did not differ significantly from controls, so that stress seems less likely to participate in the spine enlargement observed.

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% of consumed pellets		> 75	50-75	< 50
No. of animals		12	7	9
Middle Distal Third	Spines	11.49±3.67	16.85±5.35	27.26±5.65
	p	< 0.01	< 0.02	< 0.001
Proximal Third	Spines	-7.66±2.49	-2.41±4.40	4.96±6.39
	p	< 0.01	NS	NS

Table 1. Percentile differences of mean values with standard errors between controls (n=16) and conditioned mice. (Controls=100%)

725 EFFECTS OF SEPTAL AND HIPPOCAMPAL LESIONS ON PAVLOVIAN CONDITIONING OF CORNEORETINAL POTENTIAL AND HEART RATE AND BLOOD PRESSURE CHANGES. James Francis, Shirley Buchanan*, and D. A. Powell. Neuroscience Lab., VA Hospital, and Univ. of S. C., Columbia, S. C., 29201.

Rabbits received either septal, hippocampal or sham lesions and were subjected to Pavlovian conditioning training in which corneoretinal potential, electromyographic activity, heart rate, and blood pressure CRs were measured. General activity in a free field and cardiovascular and eyelid shock thresholds were also assessed in selected animals. It was found that septal as well as hippocampal lesions resulted in an enhancement of the magnitude of the conditioned heart rate response and in some animals effected a reversal of the direction of the blood pressure CR. However, heart rate discrimination was unimpaired in differential conditioning experiments. There were some indications that the acquisition of the corneoretinal potnetial CR was facilitated by hippocampal lesions, but this effect was shown to be due to the lack of latent inhibition in animals which had received prior exposure to the CS. Animals with septal lesions and hippocampal lesions also showed relatively severe impairment of the corneoretinal potential discrimination in differential Pavlovian conditioning and reversal experiments. This impairment in discrimination resulted from an increased frequency of response to the non-reinforced CS-. Extinction performance was also impaired in hippocampal lesioned animals. Assays of hippocampus and cortex in animals with septal lesions suggests that these impairments may be due to the interruption of 5HT and NE neurons in the septum in route to the hippocampus. It is suggested that 5HT and NE inputs to the hippocampus modulate hippocampal pyramidal cells and thus control the visceroautonomic response to significant stimulation (i.e., novel stimuli, or stimuli associated with a reinforcer). Such an effect on viscero-autonomic systems may result in the impairment of somato-motor systems as discussed above.

726 SPATIAL PATTERNS OF EEG ACTIVITY WITH ODORS AT SURFACE OF OLFACTORY BULB. Walter J. Freeman. Dept. Physiol.-Anat., Univ. Calif., Berkeley, CA 94720

The olfactory receptors form a surface array that projects onto the surface of the olfactory bulb through the primary nerve. There is topographic order in this projection with broad divergence, such that each local neighborhood in the bulb receives input from a subset of receptors. Adrian (1950) proposed for encoding of olfactory information that the spatially distributed receptor activity evoked by an odor might induce a spatial pattern of bulbar activity different from the pattern induced by any other discriminate odor.

The further hypothesis has been advanced (WJF, 1975) that the odor-specific information of each local neighborhood in the bulb might be reflected in the amplitude of the induced wave of EEG activity recorded at the bulbar surface above that neighborhood.

To test these hypotheses an 8x8 array of 64 electrodes was chronically implanted over the lateral surface of the olfactory bulb. The 64 signals were amplified, multiplexed, digitized at 1 msec intervals, and stored in blocks 900 msec in duration. For selected bursts of the induced wave before and during the delivery of selected odors the root mean square amplitudes $v(x,y)$ were calculated and displayed by contour plots. For each plot the values for v were normalized to zero mean and unit variance. Comparisons were made between the means of 10 plots with a certain odor A, $\bar{v}_a(x,y)$, and 10 plots without the odor, $\bar{v}_o(x,y)$, or with a different odor B, $\bar{v}_b(x,y)$.

Adaptation was overcome by pairing selected odors as CS with appetitive or aversive UCS. The means over the first conditioning session of 10 trials in each rabbit for odor A showed that $\bar{v}_a \neq \bar{v}_o$, but over the second session, $\bar{v}_a$ and $\bar{v}_o$ were not significantly different, that is, $\bar{v}_a = \bar{v}_o$. When a second odor B was given on randomly interspersed trials without the UCS, then $\bar{v}_b = \bar{v}_a = \bar{v}_o$. When thereafter A was replaced by B, then over the first session $\bar{v}_b \neq \bar{v}_a = \bar{v}_o$, but thereafter $\bar{v}_a$ disappeared and $\bar{v}_b = \bar{v}_o$. When trials with A were interspersed with trials for B, both with UCS, then over 2 or more sessions $\bar{v}_a \neq \bar{v}_b$ and either $\bar{v}_a = \bar{v}_o$ or $\bar{v}_b = \bar{v}_o$ depending on whether A or B respectively was given first on that day.

The differences between $\bar{v}_a$, $\bar{v}_b$ and $\bar{v}_o$, when they occurred, were clearly visible in contour plots. The results show that the spatial pattern of the induced EEG wave is related to the expectation of an odor rather than to the odor per se, although the odor is required to form a template for the expectation. Some implications for short-term memory mechanisms will be discussed. Supported by MH06686.

727 VISUAL MEDIATION OF ONE-TRIAL CONDITIONED AVERSION LEARNING IN CHICKS. Karen E. Gaston* (SPON: C. R. Hamilton). Division of Biology, California Institute of Technology, Pasadena, CA. 91125.

A series of experiments investigated the ability of 10-day-old domestic chicks to acquire in one trial an illness-induced aversion to the visual and/or taste properties of an unfamiliar liquid. Chicks were allowed access for one hour to a novel, distinctively colored and flavored solution (green sucrose). Several minutes after the end of this drinking session, the animals were injected intraperitoneally with an illness-inducing dose of LiCl (Experimental Group) or with isotonic saline (Control Group). In a two-choice preference test administered 24 hrs later (green sucrose solution versus plain water), the Experimental Group demonstrated a strong aversion to the green sucrose solution, whereas the Controls displayed no significant preference. Chicks trained and/or tested without the color cue (uncolored sucrose solution) failed to exhibit a conditioned taste aversion. Thus, the conditioned aversion to green sucrose was apparently mediated by the visual (color) cue.

To determine whether there is interhemispheric communication of this conditioned aversion learning, interocular transfer tests are being conducted in monocularly-trained chicks. Preliminary results suggest that when the pre-illness experience with green sucrose solution is restricted to one eye, a conditioned aversion is not demonstrated during testing with the untrained eye. This finding contrasts with the good interocular transfer observed in birds for other tasks involving color discrimination or passive avoidance, while it is similar to the lack of transfer reported for imprinting and for visual cliff habituation.

The demonstration that young chicks are able to learn in one trial an association between food color and delayed illness offers a useful new paradigm for the study of associative learning and memory processes in the avian brain, and provides new evidence which supports the view that conditioned food aversion learning is an adaptive evolutionary specialization and does not depend on the animal's "learning to learn".

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728 EFFECTS OF 6-HYDROXYDOPAMINE ON CLASSICAL (PAVLOVIAN) CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. M. P. Gimpl*, J. A. Harvey, and I. Gormezano*. Dept. Psychol., Univ. Iowa, Iowa City, Ia. 52242.

This study examined the role of the catecholamines in associative processes (learning). Rabbits (N=16) were injected with 400 µg of 6-hydroxydopamine (6-HDA) into the left and six days later into the right lateral ventricle. Control rabbits (N=16) received vehicle injections. Ten to 30 days later all animals received an adaptation session followed by 10 daily conditioning sessions. A tone or light CS (conditioned stimulus) was presented 800 msec prior to delivery of a 100 msec paraorbital shock UCS (unconditioned stimulus). There were 30 tone-shock and 30 light-shock trials each day. Extension of the membrane to the CS during the 800 msec prior to shock onset was recorded as a conditioned response (CR) and extension to UCS onset as an unconditioned response (UCR).

6-HDA produced an enhanced rate of CR acquisition. Thus, relative to controls, 6-HDA treated rabbits exhibited: a) a greater total number of CRs to both tone and light ($p<0.05$); b) fewer trials to a criterion of 10 consecutive CRs to tone (50 vs 123, $p<0.001$) and light (67 vs 132, $p<0.01$); and c) shorter latency of terminal CRs for both tone (173 vs 313 msec, $p<0.001$) and light (238 vs 367 msec, $p<0.001$).

In a separate experiment, rabbits treated with 6-HDA (N=6) or vehicle (N=6) were exposed to ten daily sessions consisting of 30 tone CS, 30 light CS and 60 shock UCS trials in an explicitly unpaired sequence to test for non-associative effects of 6-HDA. Responses of the membrane were recorded during the 800 msec of CS presentation and the 800 msec prior to UCS presentation. Although 6-HDA subjects exhibited a slightly higher rate of responding during these intervals (approximately 10%) there was no evidence for sensitization or pseudoconditioning. Thus 6-HDA appears to have a specific effect on associative processes leading to enhanced learning.

At the conclusion of behavioral testing all rabbits were decapitated and brains were assayed for content of norepinephrine (NE), dopamine (DA) and serotonin (5-HT). 6-HDA produced large and significant decreases in telencephalic content of NE (86%) and smaller though significant decreases in DA (38%) and 5-HT (28%). Brainstem content of NE and 5-HT were also significantly reduced by 37 and 19%, respectively. It is suggested that the enhanced CR acquisition produced by 6-HDA is due to the depletion of telencephalic NE. (Supported by NIMH grant MH-16841, NSF grant 6B-41531, and PHS fellowship 1F32 MH05682-01)

729 PHENOXYBENZAMINE ATTENUATION OF RETROGRADE AMNESIA. Paul E. Gold Dept. Psychology, Univ. Virginia, Charlottesville, VA 22901

The neurobiological mechanisms by which amnestic treatments act on memory are not understood. Electroconvulsive shock and direct electrical stimulation of the cortex appear to require brain seizures. Diethyldithiocarbamate (DDC), a drug which inhibits dopamine-B-hydroxylase activity, may produce amnesia by blocking brain norepinephrine synthesis. These amnestic treatments, as well as others, have a common property of being either intense physiological stressors or being themselves a component of a stress response (e.g. epinephrine or ACTH). We previously found that amnesia produced by subcutaneous posttrial epinephrine injections does not occur in animals which received a pretrial injection of phenoxybenzamine (PBZ), an alpha-adrenergic blocking agent. An agent (PBZ) which blocks amnesia produced by epinephrine may therefore also block amnesia produced by other treatments. The present study examined the possibility that PBZ might attenuate the amnestic effectiveness of frontal cortex stimulation and DDC.

Male Sprague-Dawley rats (250-300 gms) were trained in a one-trial inhibitory (passive) avoidance task. Thirty minutes prior to training, rats received injections of saline or PBZ (2 mg/kg; a dose which blocks the amnesia produced by epinephrine). After training, two groups received immediate injections of saline or DDC (680 mg/kg). Other groups received no posttrial treatment or frontal cortex stimulation (5 ma, 60 Hz, 1 sec) administered 5 sec after the training footshock (Ns=10-15). Retention performance (latency to re-enter the shock compartment; maximum=180 sec) was tested 24 hr. later.

Those animals which received saline prior to training and either frontal cortex stimulation or DDC after training had retention latencies significantly lower than those of the animals which received saline or no treatment after training. The animals which received PBZ prior to training and either frontal cortex stimulation or DDC after training had retention latencies that were comparable to those of the animals which did not receive an amnestic treatment.

These results indicate that pretrial injections of PBZ block the amnesia produced either by frontal cortex stimulation or DDC. Thus, these findings are consistent with results previously obtained using epinephrine as the amnestic agent. Because a single drug (PBZ) can block the amnestic effectiveness of these quite different treatments, the findings suggest that a common neurobiological mechanism may underlie the effects of each on memory.

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730 EFFECTS OF CHOLINESTERASE INHIBITORS ON ACQUISITION AND RETENTION OF AVOIDANCE BEHAVIOR. L. P. Gonzalez and H. L. Altshuler. Texas Research Institute of Mental Sciences and Baylor College of Medicine, Texas Medical Center, Houston, TX 77030.

Sprague-Dawley rats were trained to a criterion of 10 successive avoidances in a shuttle-box avoidance task following an injection of either saline, neostigmine methylsulphate (0.032, 0.08, or 0.32 mg/Kg), or physostigmine salicylate (0.04, 0.1, or 0.4 mg/Kg). Neostigmine impaired acquisition in a dose-related fashion. Subjects which received the lowest dose did not differ significantly from saline-injected animals, but the highest dose completely blocked acquisition. Physostigmine, in doses equimolar to those of neostigmine, impaired acquisition at all three doses. The impairment of avoidance acquisition at the lowest dose of physostigmine, but not with an equimolar dose of neostigmine, suggests the involvement of central cholinergic mechanisms in the acquisition of shuttle-box avoidance. To examine the effects of these drugs on retention of shuttle-box avoidance, animals were trained to criterion having received no drug injection and were then retrained to the same criterion following drug injection at various times after initial training (1, 7, or 14 days). Saline animals required fewer trials to reach criterion at any of the retention intervals than during original training. Neostigmine impaired performance, again in a dose-related fashion, at each of the retention intervals. The highest dose of physostigmine impaired avoidance performance at each of the retention tests. Lower doses of physostigmine, however, had a time-dependent effect upon retraining. When injected 1 day after original training 0.04 and 0.1 mg/Kg physostigmine significantly improved avoidance, but when injected 14 days after original training these doses impaired performance. The results suggest that the peripheral effects of high doses of physostigmine block avoidance performance. The effects of lower doses of physostigmine are probably due to their central actions and are dependent upon the stage of learning.

731 LOCALIZATION AND GENERALIZATION OF VISUAL MEMORY AFTER TECTAL LESIONS IN GOLDFISH Karen F. Greif* and Margaret Y. Scott* (SPON: R. W. Sperry) Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Memory for visual discrimination learning following lesions of trained tectal regions was studied in adult goldfish. Differential suppression of respiration to red or green light was used as the conditioned behavioral measure of learning (Scott, Exp. Neurol. 54: 579, 1977). The method made possible the use of small visual stimuli subtending 1.5° to be directed to precise retinal loci and hence to specifically designated target zones in the tectum. After a learning criterion was attained, the half-tectum which included the visual projections involved in training was excised. Retention was tested one day after surgery with no reinforcement in either the left or the untrained right eye.

Following training with stimuli confined to the posterior quadrant, intraretinal transfer was indicated by good differential responses from remaining untrained portions of the retinotectal system anteriorly and along the horizontal meridian. After training in the superior visual field, transfer occurred to the inferior field along the vertical meridian. The habit also generalized interocularly to the entire visual field of the untrained eye, even when initial training was restricted to the posterior-most portion of the visual field, which lies outside the region of binocular overlap. Results from 18 fish demonstrate that engrams for this discrimination task are not localized within the region of the tectum that receives direct retinal input during training. Retention of discrimination through the untrained right eye after lesion of the remaining anterior half of the right tectum suggests that engrams for monocularly-acquired visual learning are laid down bilaterally during task acquisition. Alternatively, memory traces may be stored in centers outside the tectum which remain accessible to both eyes.

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732 MEMORY LOAD AND CARDIAC INTER-BEAT-INTERVAL. Stanley W. Hall, Jr.* and J. Richard Jennings* (SPON: R. Curtis Graeber) Walter Reed Army Institute of Research, Washington, D.C. 20012

Recent work based on initial suggestions of Lacey and Lacey has supported the sensitivity of heart rate or its reciprocal, inter-beat-interval (IBI), to information processing requirements. Jennings (1975) presented evidence that cardiac acceleration might be more sensitive to a task's memory requirements than to requirements for cognitive manipulation.

The current experiment directly examined the relation between cardiac acceleration and memory by manipulating memory load between 6 and 10 items. Using a recognition memory paradigm with variable intertrial intervals, memory items were simultaneously presented for 5 sec. followed by a 5-sec unfilled retention interval terminated by a single item probe. The subject judged whether the probe was a member of the preceding set and rated confidence of judgment. A signal detection analysis of the recognition data showed the expected monotonic decrease in detection with increasing memory load.

Averaged sec.-by-sec. IBI during the memory task showed a) initial deceleration preceding presentation of the memory set, b) acceleration followed by deceleration during presentation of the memory set, and c) acceleration through most of the retention interval followed by deceleration just prior to presentation of the probe. Thus, cardiac deceleration seemed a consistent correlate of anticipating important intratrial events (ie. presentation or removal of memory set or probe) and was maintained during information input. Acceleration can be associated with times corresponding to initial storage and subsequent rehearsal of memory items. Cardiac results for correct recognitions, as compared to errors, showed less initial deceleration and less acceleration during memory set presentation, but greater acceleration during the retention interval. The exaggeration of acceleration and deceleration for error producing items during presentation of the memory set is difficult to interpret although it may represent a failure to coordinate the requirements for item input and storage.

Neither the pattern nor magnitude of IBI changes varied with memory size. Given the absence of memory load effects in the current study, cardiac acceleration cannot be interpreted in terms of memory operations. No relations between signal detection indices and cardiac change were observed, raising similar questions. Overall the results suggest that the sensitivity of IBI to information processing may arise from a general process such as the regulation of access to processing capacity rather than specific processes such as memory encoding or rehearsal.

733 OPERANT LEARNING IN THE PRECOLLICULAR HEMI-DECEREBRATE RAT: INTACT BRAINSTEM SELF-STIMULATION IPSILATERAL TO LESIONED SIDE. Joseph P. Huston and Kurt Ornstein*. Inst. Pharmacol., Univ. Zurich, Zurich, Switzerland.

Rats were imlanted with stimulating electrodes along the superior cerebellar penduncle, 1 mm lateral to the midline. The animals which self-stimulated were decerebrated unilaterally ipsilateral to the positive electrode. All brain tissue, including hypothalamus, anterior to the colliculi was removed by aspiration. Four of the animals self-stimulated by lever-pressing for stimulation delivered ipsilateral to the lesioned hemisphere. Intact self-stimulation was observed from 4 hours to 3 months after the decerebration. Extinction was comparable to intact animals. In conclusion ipsilateral ascending telencephalic and diencephalic fibers are not critical for brain-stem self-stimulation (which raises serious doubts about the significance of certain catecholamine systems that are often invoked in self-stimulation). It follows, that, if ascending projections are involved, they must be crossed fibers. It is also possible, however, that the hypothalamus and thalamus are not critical for self-stimulation (and operant learning), in addition to the rest of the forebrain, which has been shown to be dispensable in the thalamic rat preparation (Huston and Borbely, Brain Res. 1973,50:467-472).

734 CONDITIONING CAUSED BY IONTOPHORETIC APPLICATIONS OF L-GLUTAMATE ON CORTICAL NEURONES IN CONSCIOUS ANIMALS. S. Ioffe*, V. Havlicek, H. Friesen, V. Chernick (SPON: R. Jell). Depts. of Physiol. & Pediatrics, Univ. of Man., Wpg., Canada. R3E 0W3

The possibility of a role for L-glutamate as a transmitter has been widely discussed in the literature. It has been shown, for example, that L-glutamate is released from the surface of the cerebral cortex by electrical stimulation of the reticular formation as well as cortical slices (Jasper, H. et. al., 1969; Hammerstad, J.P. et. al., 1972). On the other hand, studies concerning cellular analogs of learning (Rabinovich, M.Y. et. al., 1971, Voronin, L.L. et. al., 1972) have shown that during rhythmic stimulation of the cerebral cortex if the stimulus is interrupted the neurons fire at the time a stimulus would normally have been applied. In the present experiment we tested the hypothesis that L-glutamate participates in such conditioning. Extracellular potential of 22 neurons were recorded in five rabbits. The experiments paradigm involved the application of L-glutamate (2-15 nA) for 15-20 seconds of a regularly repeating 75 sec cycle. This was repeated from 40-100 times before interruption. In 18 cells statistically significant conditioned responses ($p < 0.001$ n=18) were recorded and these sometimes equalled or even exceeded the normal response to the application of L-glutamate at the time a stimulus would normally have been applied. This data provides indirect support for a role of L-glutamate in the formation of memory traces. Supported by the Medical Research Council.

735 SHORT-TERM MEMORY: A NEUROPHARMACOLOGICALLY DISTINCT PROCESS. Stanley J. Jackson* and Herbert P. Alpern. Dept. Psych. and Inst. Behav. Gen., Univ. Colo., Boulder, CO 80309.

Since cholinergic modulation of short-term memory has been documented this investigation was undertaken to determine whether other putative neurohumoral systems are involved in the expression of short-term memory.

During the preliminary phase of the experiment subjects were initially trained to a particular position (left or right arm) of a t-maze to avoid punishing foot-shock. After mastering this problem the subjects were trained to the opposite position and then successively alternated until they could reliably reverse their behavior after a single presentation of the reversal cue (foot-shock in the previously correct maze arm). The subjects were then delayed-response tested for short-term memory. On any given day during delayed-response testing each subject was given a single trial during which the reversal cue was presented, then tested for subsequent reversal behavior 5 min. post-cue presentation. This delay interval was chosen because it invariably produces high levels of correct performance. Lastly each subject was trained to a daily 5/6 criterion. The drugs were administered to male C57BL/6 IBG mice prior to daily delayed-response testing. Subjects treated with the cholinergic blockers atropine or scopolamine demonstrated impaired performance; interestingly neither the noradrenergic blockers diethyldithiocarbamate, sotalol, propranalol, or phentolamine, or the dopaminergic blocker haloperidol, nor the serotonergic blocker p-chlorophenylalanine affected performance significantly.

The findings of this study strongly suggest that short-term memory modulation depends heavily on a cholinergic mechanism, and also suggest that short-term memory is morphologically distinct from other memory processes.

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736 AMNESIA PRODUCED BY INTRAVENTRICULAR ADMINISTRATION OF DIETHYLDITHIOCARBAMATE. Robert A. Jensen, Joe L. Martinez, Jr., Beatriz J. Vasquez*, James L. McGaugh, Teresa McGuiness*, David Marrujo*, and Scott Herness*. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

Previous work has shown that the dopamine-β-hydroxylase inhibitor diethyldithiocarbamate (DDC) produces amnesia. In addition to causing a fall in brain norepinephrine levels, peripheral administration of DDC has a number of toxic side effects that may contribute to the observed amnesia. In these experiments, DDC was administered intraventricularly to restrict the site of action and its effects on memory storage processes studied. Adult male Sprague-Dawley rats (n=271) were each implanted with a 1 cm, 25 ga stainless steel guide cannula with the tip aimed dorsal to the left lateral ventricle. Intraventricular injections of DDC (400 mg/ml; pH 11.4), NaOH (pH 11.4) or Ringer's solution were given through a 30 ga inner cannula. These injections were given either 24 hrs or 15 min before inhibitory avoidance training, or immediately, 6 hrs, or 24 hrs after training. Control animals received 10 µl injections of Ringer's solution. Additional rats received 10 µl injections of NaOH as a pH control. DDC was administered in the following doses: 4.0 mg (10 µl), 2.0 mg (5.0 µl), 1.4 mg (3.5 µl), 1.0 mg (2.5 µl), and 0.4 mg (1.0 µl). A 2-compartment black-white apparatus with a 2 mA, 2 sec footshock given upon entry to the dark side was used for inhibitory avoidance training. Three days after training, the animals were tested for retention, sacrificed, and histological examination performed.

The retention latencies of the NaOH control rats were similar to those of the Ringer's control groups in all cases indicating that the infusion of a high pH substance did not in itself produce amnesia. Profound amnesia was observed with 3 of the doses of DDC given 15 minutes before training. The median entrance latency of the rats receiving 1.4 mg DDC was 10.6 sec, 2.0 mg DDC, 1.7 sec and 4.0 mg DDC, 3.8 sec. All of these entrance latencies were shown to be significantly different from those of control animals by Mann-Whitney U-tests ($p < .01$). Only 4.0 mg DDC administered immediately after training produced amnesia ($p < .05$). All saline control groups showed median latencies of 300 sec with the single exception of the immediate post-training group in which the median latency was 297.4 seconds.

These findings demonstrate that intraventricularly administered DDC produces both anterograde and retrograde amnesia. This suggests that the amnesia seen after peripherally administered DDC is not mediated by side effects occurring outside the central nervous system. Additionally, this amnesia is both time dependent and dose related indicating that the effect of DDC is on some aspect of the memory storage process. (Supported by USPH Grants MH 05358, MH 05249, MH 12526, and NSF Grant BNS 76-17370).

737 SPECIFICITY OF PRENATAL AND PERINATAL LEARNING IN TERMS OF LATER BEHAVIOR PATTERNS: A CLINICAL FOLLOW-UP, Virginia Johnson. 1416 Westwood Blvd., Los Angeles, California 90024.

The construct of "learning" derives from the observation of adaptations or changes in behavior following an experience; if no such change can be observed, it would ordinarily be assumed no learning had taken place. Thus in animal and human newborns, certain behaviors indicate that learning already has taken place; as well as the availability of memory process. Such perinatal learning (or conditioning) has been observed in the young of several species, and it may be assumed that memory processes are activated by input from the environment prenatally as well as during and after birth.

Since such input can be demonstrated or reasonably inferred from responsive behavior in human subjects, it also follows that engrams coded at or before birth may form an early matrix to which reference is made continuously thereafter in the intact human nervous system (e.g. biofeedback). Furthermore, the nature of the input itself can be inferred from the observed behavior, thus suggesting a model for assessing the perinatal environment in terms of its effect on later behavior.

In 1971, 1972, and 1973 the author reported on various aspects of natal learning based on state-dependent experimental recall obtained under clinical conditions. The present paper summarises an analysis of this and other data which reflects specificity of early influences on later behavior, including sensory and motor expression, neurophysiological responses, and in some cases even life style. Verbal echoisms appearing spontaneously in the language of adult subjects often appeared to derive from very early learning. It is therefore suggested as a neuropsychological hypothesis in learning and memory that input from prenatal, perinatal, and postnatal environments is significant for the coding of engrams which act as experiential referrents for the developmentally older organism; and that such conditional experiences are specific and identifiable with respect to these later behavior patterns.

This model is congruent with research on neural and behavioral continuity; with clinical neurological data which relates the pathological interruption of function to specific lesions in the brain; and with the dependency of learning and conditioning upon the integrity of response systems. Furthermore, it raises the question of ethics and responsibility for the prenatal/perinatal environment in a society in which individual members may be patterned to a significant degree by early learning.

738 EFFECTS OF AMYGDALA AND STRIA TERMINALIS LESIONS ON AVERSIVE CONDITIONING IN THE RABBIT. Bruce S. Kapp, Robert C. Frysinger,* Michela Gallagher, and Andrew J. Bretschneider.* Dept. Psychol., Univ. of Vermont, Burlington, VT 05401.

In an attempt to investigate the role of the amygdala complex in learning and memory processes, we have initiated a series of studies designed at determining the effects of discrete lesions of the amygdala and its main afferent and efferent pathways on the acquisition of the nictitating membrane conditioned response in New Zealand rabbits. Rabbits received small bilateral lesions within the amygdaloid complex extending from the anterior amygdala area through the basolateral and basomedial nuclear groups. Additional rabbits were assigned to unoperated and operated control groups. Two weeks following surgery, all animals were given 100 conditioning trials per day for four successive days. Each trial consisted of a 500 msec, 92 dB, 1000 Hz tone, the offset of which coincided with the onset of a 50 msec 1.0 ma eyelid shock. The results showed that rabbits with lesions of the amygdala, when compared with operated and unoperated control rabbits, demonstrated profound deficits in the acquisition of the conditioned response, this deficit being manifested in a prolonged initial phase of non-responding.

A second experiment was designed to determine the importance of the stria terminalis, one of the two main afferent-efferent pathways of the amygdala complex, in the acquisition of the conditioned response. Rabbits receiving total or near total transection of the stria terminalis as it projects dorsally just ventromedial to the caudate nucleus did not demonstrate deficits in acquisition of the conditioned response when compared to unoperated control rabbits. The results of these experiments suggest that the amygdala complex and/or the afferent-efferent fibers projecting via the ventral amygdalofugal pathways play an important role in the acquisition of the nictitating membrane conditioned response.

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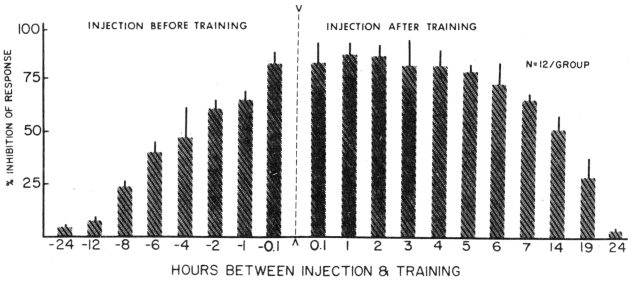
739 **ANTISERUM TO BRAIN GANGLIOSIDES INHIBITS CONSOLIDATION PHASES OF LEARNING.** **S. Karpiak, T. Sowin*, and M.M. Rapport, Div. Neuroscience, N.Y. State Psychiatric Inst., New York, N.Y. 10032.**

Intraventricular (i.ventric.) injection immediately after training (on a passive avoidance learning paradigm) of antiserum to brain gangliosides causes >90% inhibition of the learned response (Neurosci.Abstr. 2: 443). As a control, rats injected with antiserum after absorption with pure G_{M1} ganglioside show no inhibition. We have now examined the temporal parameters of the effects of i.ventric. injection of antiserum to brain gangliosides on learning. Mice (groups of 12) were injected with 8 µl of antiserum to total brain ganglioside at intervals before and after training on a step-through passive avoidance task. They were retested for the learned response 7 days after training. The results (Fig) show that with the shorter intervals between injection of antiserum and time of training, the inhibition of the learned response was greater. A high degree of inhibition (>75%) occurred with injection as long as 6 hrs after training. The control antiserum absorbed with G_{M1} ganglioside injected either at 4 hrs or 1 hr prior to training, or either 5 min, 1, 4 or 14 hrs after training caused no inhibition. Interferences with the learning process produced by antiserum injected before and after training were different since no inhibition was found with the 12 hr interval for injection preceding training (Fig). To determine whether the antiserum to ganglioside was affecting the acquisition or consolidation phases of learning, independent groups of mice were tested one hr (rather than 7 days) after injection, with intervals of 5 min, 1, 4 and 14 hrs between training and injection. These animals showed no inhibition. The results indicate that the antiserum inhibited the consolidation phases of learning and not the acquisition phase.



740 RELATIONS BETWEEN BEHAVIOR, SINGLE-UNIT ACTIVITY IN THE MIDBRAIN RETICULAR FORMATION, AND HIPPOCAMPAL THETA ACTIVITY IN THE ACTIVE RAT. John Kootz and Harry M. Sinnamon (Spon: D.B. Adams), Laboratory of Neuropsychology, Wesleyan University, Middletown, Ct. 06457.

Rats were prepared for recording of single-unit activity in the midbrain reticular formation (MRF) by means of movable microelectrodes and for recording of EEG activity in the hippocampus by means of fixed bipolar electrodes. Following recovery, tests for habituation, dishabituation and sensitization of orienting and movement elicited by click and flash stimuli were made. In preliminary tests for sensory responsiveness, the group of 24 units in the MRF were found to be particularly sensitive to tactile stimuli and to show more multimodal responses than non-MRF sites. Significant correlations, only one of which was negative, between behavior elicited by novel stimuli and unit activity were found for 13 of 24 units in the MRF. However, during tests for habituation and sensitization, unit activity in the MRF and behavior often failed to correlate. Only 4 of 13 MRF units recorded during behavioral havituation showed a parallel decrease in activity, and even in these cases, several instances of behavior-unit disassociation were found. Similar disassociations occurred during behavioral sensitization. Hippocampal theta activity, although moderately correlated with behavior in most animals was correlated with activity in only four MRF units. Activity in the MRF seems to bear no simple, close relationship with behavior elicited by novel stimuli.

741 GENETIC DIFFERENCES IN CYCLOHEXIMIDE INDUCED MEMORY IMPAIRMENT AND PROTEIN SYNTHESIS INHIBITION. Neal R. Kramarcy and Elton E. Quinton. Dept. Psych., Univ. of Louisville, Louisville, Kentucky 40208

Two strains of mice (C57BL/6J and DBA/2J) that have different memory and neurochemical characteristics were administered cycloheximide before training on a passive avoidance task. Retention was tested 72 hours later. Doses of cycloheximide as low as 7 mg/kg were effective in producing amnesia in the C57BL/6J when given 30 min. before training, and 150 mg/kg was amnesic up to 90 min. before training. The degree of memory impairment was both dose and time dependent in the C57BL/6J. In contrast, the DBA/2J exhibited memory impairment only after 150 mg/kg given 30 min. before training. Measurement of cerebral protein synthesis up to 6 hours after cycloheximide indicated a dose dependent relationship for the degree of inhibition and the rate of recovery in both strains. These data further suggest that memory-specific protein synthesis recovers more rapidly from cycloheximide than does general cerebral protein synthesis. Cerebral protein synthesis recovered more rapidly from cycloheximide in the DBA/2J than in the C57BL/6J. These results suggest that the difference in the effectiveness of cycloheximide on memory in the two strains may be due to the more rapid recovery of protein synthesis in the DBA/2J.

742 SODIUM NITRITE INDUCED AMNESIA IN RATS AND MICE. Joe L. Martinez, Jr., Robert A. Jensen, Beatriz J. Vasquez*, Joe S. Lacob*, and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

We investigated the effects of sodium nitrite ($NaNO_2$) on acquisition and consolidation of an inhibitory avoidance task. $NaNO_2$ is a compound that has two distinct and specific actions. The first is to cause a relaxation of smooth muscle. Thus, one major site of action of $NaNO_2$ is vascular smooth muscle; $NaNO_2$ causes vasodilitation which may result in a hypotensive state. Secondly, the nitrite ion oxidizes hemoglobin to methemoglobin (MHb), and in high doses, may impair the oxygen carrying ability of red blood cells. These effects should produce hypoxia depending on dose. Most experimental studies induce hypoxic amnesia by administering gas mixtures. Surprisingly, little data are available on hypoxic amnesia induced through pharmacological treatment.

Male Swiss-Webster mice and Sprague-Dawley rats (50-60 day) were trained in a one-trial inhibitory avoidance step-through task. Animals were placed in a well-lit start compartment. Upon stepping through to the dark side, they received a footshock. Rats received a 2 mA, 2 sec inescapable footshock while mice received a 350 μA footshock terminated by escaping back to the start chamber. A retention test was given either 48 hrs (rats) or 72 hrs (mice) after training. The cutoff latencies were 300 sec (rats) and 600 sec (mice). The $NaNO_2$ was dissolved in distilled water and administered to the animals either 30 min (mice) or 15 min (rats) before training or immediately after training in the following doses: Mice (0.10, 1.0, 10.0, 100.0 mg/kg, i.p.); rats in the pretraining administration condition (5.0, 25.0, 50.0 mg/kg); rats in the posttraining condition (0.5, 5.0, 50.0 mg/kg). Significant differences resulted only when the animals were treated before training. Thus, for rats 5.0 and 25.0 mg/kg $NaNO_2$ produced amnesia (U=770.5, Z=5.915, $p<.0001$; U=733, Z=1.96, p=.05). For the mice only one dose (0.10 mg/kg) produced amnesia (U=859, Z=2.91, p=.0036). The effect was inversely dose related since in no case did the highest dose produce amnesia. MHb levels in $NaNO_2$-treated rats were determined by a cyanmethemoglobin assay. The following percentages of MHb were found following $NaNO_2$ administration: saline=0%; 0.5 mg/kg=0%; 5.0 mg/kg=2.0%; 25.0 mg/kg=19.49%; 50.0 mg/kg=33.25%. Additionally, in rats we found no gross EEG abnormalities such as epileptiform activity associated with a single dose of $NaNO_2$.

These findings indicate that $NaNO_2$ produces anterograde amnesia. However, the amnesia is not strictly dose-related, indicating that compensatory mechanisms were inititated by higher doses of $NaNO_2$. (Supported by UPHS grants MH 05249, MH 05358, MH 12526, AG 00469, and BNS 76-17370).

743 INSTRUMENTAL LEARNING IMPAIRMENT FOLLOWING LESIONS OF VENTRAL ANTERIOR THALAMUS IN RAT. Darryl B. Neill* and Michael D. Britt* (SPON: David Freides). Dept. Psychology, Emory Univ., Atlanta, GA 30322

Electrolytic lesions were bilaterally placed in the thalami of rats to include the ventral anterior and ventral medial nuclei. These animals were found to be impaired in the acquisition of a number of instrumentally-learned responses. They had difficulty learning to bar-press for food or water, and to run an alley for food reward. In addition, they could not learn to perform a 1-way avoidance response in an alley to avoid shock. A number of experiments were conducted to assess the nature of the impairment. First, if trained preoperatively on alley-running or bar-pressing for food or on alley shock avoidance, subsequent lesioning did not reliably affect performance on any of the tasks tested, indicating specificity for acquisition. Second, both spontaneous and amphetamine-induced locomotor activity were normal, indicating little or no impairment in gross motor function. Third, any lesioned-induced hypophagia or hypodipsia was limited to 2-3 days post-surgery, and the animals' food and water intakes appeared normal thereafter. When food or water-deprived, they immediately consumed food or water presented in the home cage. Fourth, the emotional reactions to handling and shock of the lesioned rats appeared normal. It was concluded that the learning impairments observed could not be readily explained on the basis of changes in gross motor ability, incentive value of reward, or emotionality.

This thalamic area is at the confluence of a number of cellular and axonal systems which have recently been linked to instrumental conditioning. The ventral medial nuclei of the rat are terminal sites of the nigrothalamic projection. The ventral anterior and medial nuclei receive a heavy projection from the globus pallidus. Silver impregnation studies of the forebrains of rats revealed major degeneration sites in dorsolateral anterior neocortex and neostriatum.

In summary, we feel that this thalamic region may contain axons and/or cell bodies involved in instrumental conditioning. Although our experiments tend to rule out some explanations, the exact nature of this involvement remains to be defined.

744 PONTINE RETICULAR FORMATION NEURON ACTIVITY DURING CLASSICAL CONDITIONING. Robert J. Norman and Jennifer S. Buchwald, Dept. of Physiology, Brain Res. Inst., Sch. Med., UCLA, Los Angeles CA, 90024.

We have previously reported conditioning of the blink response in chronic decerebrate cats implicating the pontine reticular formation as the site supporting such learning. In this study, single unit activity from the pontine reticular formation was recorded during classical conditioning with auditory discrimination in normal and bilaterally hemispherectomized cats. The cats were restrained in an atraumatic head holder allowing stereotaxic placement of metal microelectrodes into various location in the reticular formation and medial thalamic sites in the awake and behaving animal. Conditioning trials consisted of the presentation of a conditioned stimulus (500 msec., 65 db SPL) followed by a single shock to the outer canthus of the left eye (2 msec., 30 v). EMG was recorded bilaterally from orbicularis oculus. The discrimination procedure consisted of presenting tones differing in frequency from the conditioned stimulus but without the shock. Following several hundred trials the cats would respond with a characteristic conditioned response to the reinforced tone (CS+) but more infrequently or not at all to the unreinforced tone (CS-). Unit recordings were made only when the behavioral response had been well stabilized to avoid the more transient correlates of learning associated with the acquisition phase of conditioning. Unit activity was analyzed separately for four classes of trials depending on whether or not the animal responded and whether or not a response was appropriate on that trial (CS+, CS-). Correlations were also obtained for EMG reflex components which often occur during conditioning trials but which are not considered to be an essential part of the conditioning process. In contrast to the unconditioned state, a large percentage of neurons encountered showed reliable responses to the stimuli used in conditioning. Analysis of the patterns of activity suggested that the unit responses are more closely related to the motor response than to sensory input. Cross correlation of unit onset latency with EMG latency on conditioning trials suggests that the RF units are leading the conditioned response and represent a component of the central initiation of the response. (Supported by USPHS Grant NS-05437).

745 ASSOCIATIVE AND NON-ASSOCIATIVE CHANGES IN AUDITORY SYSTEM UNIT RESPONSES DURING CLASSICAL CONDITIONING IN THE RAT. J. Olds, R. Nienhuis* Div. of Biol., Calif. Inst. of Tech. Pasadena, CA 91125

Experiments were conducted to localize unit changes of short latency in the medial geniculate body and in the inferior colliculus of the rat during differential classical conditioning tests with tones as the stimuli and food as the reward. In an earlier study changes in responsiveness of an abrupt character appeared when the experimental paradigm shifted from the random presentation of signals to the paired conditioning trials. This observation indicated the possibility of arousal and orienting factors as the source of some of the changes. It seemed possible that the different average position of the CS+ in relation to the reward in conditioning trials as compared to the pseudoconditioning trials might be responsible for these changes. During the random series it was at a midpoint in the pellet-pellet interval; in the conditioning trials it was at the end of the pellet-pellet interval. In the first experiment to test for arousal effects, responses of 52 inferior colliculus and medial geniculate body units in 8 freely moving rats were studied at each of 8 randomly varied intervals (ranging from 20 sec to 180 sec following a food pellet). Large changes with a linear trend over the 8 intervals were observed in the initial onset component but not the later components of the unit responses. The changes consisted of two components, increases in the peak-response firing, and decreases in spontaneous background firing. It was therefore concluded that such changes could have been an artifact in earlier differential conditioning studies. Therefore, in the second experiment additional restrictive criteria for associative changes were instituted. 119 units in medial geniculate body and 135 units in inferior colliculus were studied during a differential classical conditioning task, in order to assess the associative nature of the later components of the auditory response. Restrictive criteria for associative changes were applied to individual cases and were aimed to eliminate previously uncontrolled-for, non-associative changes. Associative changes with latencies ranging between 25-57 msec were observed in 14 units in the medial part of the medial geniculate body and in the posterior nucleus. Changes with shorter latencies were observed but they did not meet the restrictive criteria. Responsiveness during conditioning was characteristic for different parts of the medial geniculate body. No clear associative changes were seen in the inferior colliculus.

746 MNEMONIC DISTURBANCE IN MACAQUES FROM STIMULATION OF ANTERIOR COMMISSURE VERSUS LIMBIC SYSTEM OR BASAL GANGLIA. William H. Overman, Jr.* and Robert W. Doty, Center for Brain Research, University of Rochester, Rochester, New York 14642.

Previous work (In: Lateralization in the Nervous System, Harnad, et al., Eds., Academic Press, 1977) had shown that electrical stimulation of the anterior commissure (AC) eliminated manual responses to conditional stimuli in 3 highly trained macaques. This effect was tentatively attributed to amnestic rather than to motoric or ictal causes since the animals displayed no disturbance of spontaneous movement and could respond promptly after cessation of tetanization of AC. In order to specify more precisely the nature of the disruption, 3 additional macaques were trained on a delayed match-to-sample task in which they selected (matched) from a pair of 100 visual stimuli, 50 trials per day, that stimulus (sample) which had been presented 5 sec earlier. Average accuracy was 94% correct. Tetanization with 0.2-msec pulses, 0.6 mA, 50 Hz, was then applied for 4 sec through electrodes implanted at one of 45 (histologically verified) loci during either the "sample", "delay", or "match" period for 25-35 trials each, interspersed among some 2000 control trials. Tetanization of AC produced no evidence of electrical after-discharge, but reduced accuracy of response to chance levels when applied during the "sample" or the "match" period ("match" responses made immediately after cessation of tetanization). Application during the "delay" was without effect. Tetanization of caudate nucleus, putamen or globus pallidus produced a similar failure to respond during the "sample" or "match" period, but generally did not interfere with accuracy of response, even in some instances in which intensities were used which could produce localized electrical after-discharge. On the other hand, stimulation at other loci in basal ganglia in some instances did reduce the accuracy of responding, and evidence of after-discharge was common. Tetanization in hippocampus or fusiform gyrus usually reduced accuracy of response to chance levels regardless of when the stimulation occurred, and whether or not localized after-discharges could be recorded using the currents employed in the behavioral situation. Stimulation at many loci, e.g. splenium of corpus callosum or superior temporal gyrus, was without effect on performance, although the animal often appeared to detect the stimulation. Stimulation of midline AC was ineffective in one case in which it was inadvertently transected by an hemorrhagic electrode track 5 mm lateral to the midline, and in another instance when the electrode barely contacted the posterior surface of AC. The primary conclusion is that tetanization of the intact AC effects a bilateral, transitory disruption of access to memory traces.

747 BRAIN-STIMULATION AS THE DISCRIMINATIVE STIMULUS IN A LITHIUM CHLORIDE CONDITIONED AVERSION PARADIGM. Anthony G. Phillips and F.G. LePiane*. Dept.Psychol., Univ. of British Columbia, Vancouver, Canada.

Electrical stimulation of basolateral amygdala during or immediately after consumption of a novel saccharin solution disrupts conditioning of taste aversion that normally occurs following subsequent poisoning with LiCl (0.2 mol. x 2% body weight). This effect appears to be locus specific as stimulation of neostriatum or substantia nigra failed to disrupt conditioned taste aversion although comparable stimulation will interfere with retention of a passive avoidance response. In an attempt to discover the nature of this disruptive effect on conditioned taste aversion, stimulation of basolateral amygdala or neostriatum was paired with drinking of H_2O prior to poisoning with LiCl. Pairing amygdaloid stimulation with H_2O in the 10 min drinking period. 48 h after LiCl treatment resulted in a 60% decrease in volume consumed. Caudate stimulation had no significant effect. Electrical stimulation of either nucleus had no significant effect on H_2O intake during the conditioning trial. Water intake 24 h after pairing LiCl with brain stimulation, was also unaffected. Injection of NaCl during the conditioning trial had no significant effect on subsequent H_2O intake in both groups of stimulated control animals. Similarly, injections of LiCl failed to disrupt H_2O intake, 24 and 48 h later, in unstimulated controls with electrodes implantated in basolateral amygdala or neostriatum. These results clearly show that stimulation of basolateral amygdala can serve as a discriminative stimulus in a LiCl aversion paradigm. Consequently, pairing amygdala stimulation with novel taste may serve as a complex conditioned stimulus in a conditioned taste aversion experiment. Therefore, apparent disruption of taste aversion during retention tests in which brain stimulation is omitted can best be attributed to failure to provide subjects with the original complex conditioned stimulus.

748 LINGUISTIC SPECIALIZATION OF THE LEFT HEMISPHERE. Francis J. Pirozzolo*, Harry A. Whitaker, Ola A. Selnes*, and Frederick Horner*. Department of Psychology, University of Rochester, Rochester, New York 14627.

Several recent attempts have been made to assess the auditory language capacity of children who have undergone left hemispherectomies early in life as neurosurgical treatment for Sturge-Weber-Dimitri Syndrome. Although the enforced development of speech and language in the right hemisphere seems to be normal by certain gross measures (e.g., verbal IQ), the right hemisphere does not appear to be a good substrate for the development of certain specific language skills such as syntactical competence. In relation to right hemispherectomized patients, these children exhibit deficiencies in repeating stylistically permuted sentences, integrating syntactic features to replace missing pronouns, and recognizing and correcting syntactically anamolous sentences. These results suggest that the two cerebral hemispheres may not be ontogenetically equal in their ability to serve as substrates for certain linguistic functions.

Using special tests designed to measure syntactic competence in a nine year-old boy with asymptomatic agenesis of the anterior two-thirds of the left temporal lobe (diagnosed by CT scan and confirmed at neurosurgery), we present further evidence of the role of the temporal lobe in language functions and the inferiority of the right hemisphere in performing at a level equal to the left in language acquisition. Although this patient had a WISC-R IQ of 121, he scored in the first percentile on a standardized test of receptive syntactical ability. Other auditory-linguistic defecits were revealed by our battery of language tests and they were incongruous with results of tests designed to measure spatial abilities. This case of agenesis of the left temporal lobe, along with other clinical and experimental observations, may provide compelling evidence to support the notion that the left hemisphere is genetically specialized or pre-programmed to perform language functions such as the auditory analysis of speech sounds.

749 CONCOMMITANT SOMATIC AND CARDIOVASCULAR CONDITIONING: EFFECTS OF CAUDATE LESIONS. D. A. Powell, Donald Mankowski*, and Shirley Buchanan*. Neuroscience Lab., VA Hospital, and Univ. of S. C., Columbia, S. C., 29201.

Different groups of rabbits received either unilateral or bilateral caudate lesions or were sham operated. Two weeks later all animals were subjected to simple or differential Pavlovian conditioning in which 500 millisec. tones of 80 db SPL intensity served as the CS and 200 millisec., 5Ma paraorbital shock served as the UCS. Corneoretinal potential (CRP), electromyographic activity (EMG) and heart rate (HR) CRs were assessed. In control experiments the unconditioned HR response as well as the CRP threshold to different intensities of shock was measured. Free field activity was also assessed. It was found that rabbits with bilateral caudate lesions, which destroyed 2/3 of the anterio-medial head of the caudate nucleus, severely impaired the acquisition of the Pavlovian conditioned corneoretinal potential response. Animals with smaller lesions (less than 1/3) or unilateral lesions showed normal acquisition of the CRP CR. However, large bilateral caudate lesions had no effect upon either the magnitude of the heart rate CR or the heart rate discrimination. Thus animals with relatively large lesions of the head of the caudate nucleus revealed a severe impairment in somatomotor learning but visceroautonomic conditioning was intact. Control experiments showed that generalized electromyographic activity was unaffected, as was general activity in a free field. Neither the CRP thresholds to shock or the unconditioned heart rate response was affected by caudate lesions. These data thus suggest that bilateral caudate lesions result in a specific impairment in the ability to generate a learned somatomotor response but do not impair visceroautonomic accompaniments of somatomotor learning.

750 ESCOPOLAMINE INJECTIONS INTO THE CAUDATE NUCLEUS. CORRELATION BETWEEN DEGREES OF TRAINING AND INSTRUMENTAL BEHAVIOR. Roberto A. Prado-Alcalá, Patricia Kaufmann Carbia* and Renee Moscona Alasraki*. Dept. Psychophysiol. Sch. Psychol. Anáhuac Univ. and Physiol. Dept. Med. Sch. Ntnl. Univ. of México, México City, México.

At last year's NS meeting (Prado-Alcalá and Cobos-Zapiaín, NS Abstracts, Vol. II) we showed that cholinergic blockade of the caudate nucleus (NC) of cats produced a significant impairment in the performance of recently acquired instrumental behaviors, whereas overtrained responses remain unaffected. In order to further study this effect we injected escopolamine into the CN of rats with different degrees of training.

Several groups of Wistar rats were trained under a CRF schedule to press a lever in order to be reinforced with water, during 5, 15 or 25 daily sessions. After training some were bilaterally implanted with cannulae in the CN. Two days after surgery they were retrained, and before the 4th session they were injected through the cannulae with a escopolamine bromide solution (30 ug/NC) or with saline solution. Unimplanted rats were trained for an equivalent number of sessions.

As compared with the unimplanted and the saline groups, it was found that escopolamine treatments induced: a) a highly significant impairment in instrumental performance in the 5-sessions groups; b) a mild effect in the 15-sessions group, and c) no deficits in the overtrained group.

These data confirm our earlier finding, and together with recent observations, support the hypothesis that as training progresses cholinergic mechanisms within the caudate nucleus become less involved in mediating instrumental responding, or that the CN, as a whole, plays a less significant role in such behaviors.

751 AGE DIFFERENCES IN ELECTRODERMAL AND DECISION RESPONSES DURING RECOGNITION MEMORY TESTING. Walter H. Riege, Howard F. Wallach* and Michael J. Cohen*. VA Hospital Sepulveda, CA 91343 and Dept. Psychiatry, Sch. Med., UCLA, Los Angeles, CA 90024.

Electrodermal responses (EDR) frequently are taken as an index of autonomic activity related to cognitive processing. Thompson and Marsh (1973, 1977) postulated a more restricted range in autonomic responsivity for the old in contrast with the young. To investigate correlation of EDR with decision and retrieval from semantic memory, we measured amplitude changes of skin potential (SP) and skin conductance (SC) time-locked with a person's decision responses (Yes/No) and decision time in a verbal recurrent recognition task. We predicted that the proverbial slower decision making of the old would produce lower EDR and more decision certainty.

Male volunteers (N = 12/group) from three age groups (Young, mean age $\bar{x}$ = 24.7 years; Middle-Aged, $\bar{x}$ = 44.4; Old, $\bar{x}$ = 65.9) were asked to selectively remember 16 words (8 emotionally charged, 8 neutral words selected from the Polarization Dimension, Heise, 1965) and to recognize these in lists of 50 words, equally balanced for emotional and neutral charge. Words were presented via tape recorder in fixed random order at a rate of one word every 20 sec while EDR and decision time (interval from repeating the presented word to its recognition decision) were recorded.

We found that the amplitude changes of EDR's were larger and the decision times shorter for emotionally charged than for neutral words. Correspondingly, more charged than neutral words were recognized by all persons. The old, on the other hand, made fewer correct and fewer false recognitions and had, unexpectedly, neither smaller SP nor smaller SC changes than younger persons. The old did also not take more but less time to decide; they adopted higher decision criteria (Signal Detection C_x), but these measures were neither correlated with EDR nor with decision time data. Only the middle-aged, not the old, had low EDR changes. In fact, EDR changes correlated with correct recognition only for neutral words in the young (4 = 0.44) and the middle-aged (r = 0.65) and did not differ for to-be-remembered or background words, so that we conclude electrodermal activity reflects general task effort rather than autonomic reactivity related to decision or memory processing.

752 THE ROLE OF ASSEMBLIES OF NEURONS IN A THEORY OF MEMORY. Kathleen J. Roney* and Gordon L. Shaw. Physics Department, University of California, Irvine, California 92717.

A study of the memory storage capacity of a network of N neurons having Hebb-type modifiable synapses (Shaw, "Space-Time Correlations of Neuronal Firing Related to Memory Storage Capacity," to be published) was made, based on an analysis of a model proposed by Little and Shaw (Behav. Biol. 14: 115-133, 1975). Although there are 2^N possible firing patterns for the N neurons in a given time step, it was found that only N linear combinations of these patterns dominate the firing behavior of the network. In response to a stimulus, a time sequence of these combinations will be excited. The sequence describes the average firing behavior as a function of time for each neuron in the network following stimulation and can be identified with the post stimulus histogram (PSH) for a given neuron. Using the definition of an assembly of neurons as hypothesized by John (Science 177: 850-864, 1972), the PSH of one neuron in an assembly (i.e., the response of a single neuron averaged over many presentations of the same stimulus) is equivalent to the average firing for all the neurons of an assembly in response to a single presentation of the stimulus. Thus, by applying John's hypothesis, we find that a highly interconnected network can be excited into many different sequences of (averaged) firing patterns of neuronal assemblies. This allows the response of a network to a single stimulus to be reproducible, as it must be in order to correlate with behavior. Thus the cell assembly concept is central to the analysis and appears physiologically reasonable in that an assembly of neurons permits the loss of some neurons without jeopardizing a particular firing pattern sequence, thereby preserving the memory associated with that particular sequence. To test these results, experiments involving the use of two or more extracellular microelectrodes must be done. In anticipation of the possible experimental confirmation of the existence of assemblies, we consider various possible physiological bases for the necessary "instantaneous" averaging over an assembly (e.g., electrotonic synapses). We will present results on the use of this physiological realization of John's hypothesis to explicitly incorporate assemblies into the theory.

753 HORMONAL INFLUENCES UPON SYNAPTIC PLASTICITY UNDERLYING THE KINDLING PROCESS. Robert P. Rose*, Frank Morrell and Thomas J. Hoeppner. Dept. of Neurological Sciences, Rush-Presbyterian-St. Luke's Med. Ctr., Chicago, Il. 60612 and Dept. of Neurosciences, Albert Einstein College of Medicine, Bronx, N.Y. 10461

"Kindling" is a progressively augmenting convulsive response to intermittent focal electrical stimulation of brain. A durable alteration of synaptic function is thought to underlie this process. Substantial evidence points to the importance of hormonal influences upon synaptic plasticity in learning situations. This study examines possible hormonal influences in kindling.

Untreated (control) and hypophysectomized male albino rats bearing implanted depth electrodes in basolateral amygdala of one side were subjected to daily electrical stimulation (150 uA, 60 Hz., 1 msec pulses, 2 sec. train duration). Polygraph recordings of cortical (via calvarium screws) and amygdala activity were made before and after stimulation. After-discharge durations and all behavioral manifestations of seizure activity were noted. Daily stimulation was continued until animals manifested tonic-clonic convulsions on three successive days. Animals were sacrificed after kindling was completed, and histology was performed (cresyl violet) to confirm electrode localization.

Hypophysectomy resulted in marked changes in the kindling process. If stimulation was initiated within two weeks of hypophysectomy the kindling rate was slowed. If stimulation was delayed more than three weeks kindling developed more rapidly than in controls. In both control and hypophysectomized animals the process was influenced by systemic steroid administration, suggesting that the effect of hypophysectomy was hormonally mediated. The findings suggest that hormonal mechanisms may play a role in the neural plasticity underlying kindling.

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754 LOCAL LEARNING IN CORTICAL NEURONS. Stuart M. Rosenblum*, M. Byron Wilder*, and James H. O'Brien. Department of Medical Psychology, University of Oregon Health Sciences Center, Portland, Oregon 97201.

Attempts to understand the neural basis of learning have often involved a search for the sites within the brain at which learning occurs. In recent neurophysiological approaches, a demonstration of sites of local learning has amounted to differentiating between those neurons which actively participate in learning and those which are passively involved. Two experiments described in this presentation have shown that local learning changes occur in the sensory-motor cortex of the cat. All experiments were performed in acutely prepared, awake, immobilized cats.

Experiment One. Conditioned responses developed in pyramidal tract cells from pairing a sensory afferent stimulus (CS) with stimulation of the pyramidal tract which antidromically activated the cells (US). The results of a control experiment indicated that the conditioned changes in the response of the cortical neuron did not develop if the magnitude of the US was set just below the threshold to produce an antidromic spike in the recorded neuron. The learning changes were clearly not due to any orthodromic afferent systems activated by the US. Thus, it was concluded that the cortical conditioned response was not projected from some other locus within the brain but was mediated locally within the cortex.

Experiment Two. A reversible cryogenic blockade of the anterior midline thalamus was introduced during the middle one-third of a classical conditioning sequence. This blockade of nonspecific afferent input to the cortex resulted in disruption of cortical neuronal conditioning both during and following the cooling. The level of conditioned responses following termination of the cooling was significantly less than that found without cooling. This demonstrated that the conditioning which was found for neurons at the cortical level was not simply a reflection of changes which took place peripheral to the cryogenic blockade of the thalamus. These experiments provide evidence that the neurons of the cat sensory-motor cortex are capable of actively mediating the elaboration of a conditioned response, and so constitute a site of local learning within the brain.

755 RETICULAR STIMULATION FACILITATES RETRIEVAL OF MEMORY AFTER AN EXTENDED TRAINING TO TEST INTERVAL. Susan J. Sara, Bernard Deweer° and Bernard Hars°. CPEC, Univ. Louvain, 3041 Pellenberg Belgium, and Dept. Psychophysiologie, LPN, CNRS, GIF/Yvette,France

Stimulation of the mesencephalic reticular formation at a very low intensity has been used to facilitate treatment of information acquired immediately prior to the stimulation (Bloch and Deweer, 1968). The contiguity between the original presentation of information and the stimulation seems to be essential, as it is in most studies of hypermnestic and amnestic effects of physiological manipulations. However, Sara and Remacle have recently shown (BB, 1977) that increasing central activation by pretest injections of strychnine can facilitate retrieval. The present study investigated the effect of increasing arousal by reticular stimulation on retrieval.

Rats were given partial acquisition of a complex maze task and tested for retention 30 days after the first acquisition trial. Half of the rats were submitted to a reactivation (reminder) treatment immediately before retention testing and half were not reminded. These groups were subdivided into stimulated and non stimulated groups, in order to study the effect of the stimulation under different cognitive contexts. Results showed that the rats which were neither reminded nor stimulated showed a performance decrement compared to their last acquisition trial (forgetting). They took longer to run the maze and more errors than at acquisition. The reminder procedure alleviated forgetting when measured either by run time or errors. Reticular stimulation diminished the number of errors, but not run time, suggesting that memory retrieval is a multifaceted process and that these two treatments facilitate different aspects of this process.

A second test, twenty-four hours after the initial one using the same pretest manipulations , showed that there were no longer any differences among treatment groups. This was taken as further evidence that the forgetting seen in the non reminded, non-stimulated control group was a lapse, not a loss and that the forgetting is due to retrieval failure. Furthermore, retrieval is shown to be a labile process, subject to physiological interventions, thus establishing this aspect of memory as a potentially fruitful area of research, which has not, as yet been widely explored by psychobiologists.

756 EFFECT OF ANTISERA TO β AND γ GOLDFISH BRAIN PROTEINS ON THE RETENTION OF A NEWLY ACQUIRED BEHAVIOR. Victor E. Shashoua and Mary E. Moore*. McLean Hospital, Mailman Res. Ctr., Dept. of Biol. Chem., Harvard Medical School, Belmont, MA 02178.

In previous studies (PNAS 74, 1743-1747, 1977) three brain proteins were identified by double labeling methods as products which incorporated more labeled valine, after goldfish acquired a new pattern of behavior. Two of these proteins (β and γ) were subsequently isolated and used as antigens to prepare antisera in rabbits (Brain Res. 122, 113-124, 1977). The monospecificity of the antisera was established by several criteria including their capacity to precipitate the labeled antigens from total goldfish brain cytoplasmic proteins. The β and γ antisera were injected into the 4th ventricle of the brains of trained animals at various times after the initiation of training. Goldfish (98 animals) injected with antisera between 8 hours and as long as 48 hours after the initiation of training, could not recall the behavior when tested 3 days after training. Trained goldfish (97 animals) receiving nonimmunized rabbit serum were used as controls; they had complete recall of the behavior. Also no amnestic effects were obtained when goldfish were injected with an antiserum to a neuronal surface membrane protein NS-6 (obtained from Drs. J. Chaffee and M. Schachner of the Children's Hospital, Harvard Medical School). This suggests that not every antiserum to a brain specific protein can inhibit the recall of the training. In addition, the possibility that the antisera were toxic was controlled for by the observation that injections of β and γ antisera, prior to the training, produced no effects on the rate of acquisition or recall of the behavior. A study of the residence time of the antisera in the brain following an intraventricular injection was also carried out. The results are consistent with the hypothesis that β and γ might have some direct role in one of the biochemical steps leading to the formation of a long-term memory. (Supported by grants from NINCDS #09407 and The McKnight Foundation.)

757 PAVLOVIAN DEFENSIVE CONDITIONING IN 1 WEEK, 4 WEEK, 12 WEEK OLD KITTENS AND ADULT CATS. S. Stefan Soltysik* and George Wolfe* (SPON: N.A. Buchwald), MRRC, Sch. of Med., UCLA, Los Angeles, CA. 90024.

Hindleg flexion, running, vocalization, respiratory and heart rate changes were recorded during classical defensive conditioning in cats and kittens. The subjects were trained in a specially designed Pavlovian stand in which the treadmill floor enabled running with the animal's head immobilized. Most of the subjects had cranial implants for electrophysiological recording from the brain. EKG was taken with chest electrodes. Respiration was monitored with a small thermistor positioned in an external nare. Vertical and forward-backward movements of the left foot were recorded by means of jointed levers. The Conditioned Stimulus (CS) was a 5.25 sec steady stream of air from a 15 gauge tube directed at the sacral region. The shock Unconditioned Stimulus (US) from an AC stimulator lasted 300 msec and started 5 sec after the CS onset. Training consisted of 10 sessions of 10 trials each run twice a day for 5 consecutive days (100 trials of CS-US pairing). The training was preceded by a 5 trial session of CS alone and followed by two 20 trial extinction sessions.

Leg flexions to CS did not develop in 1 and 4 week old kittens, but were fairly evident at the age of 12 weeks (>60% in sessions 9 and 10) and even more frequent in adults (>80% in sessions 9 and 10). Conditioned vocalization was typical in adults and in some of the 12 week old kittens. Two types of vocal responses were observed. Conditioned distress calls were correlated with stable leg flexion responding to CS; angry growls were observed in cats with poorer performance of the conditioned leg flexion. Running was not strengthened or maintained by CS-US pairing and disappeared during training. Heart rate and respiratory changes to CS were present at all ages. Several types of cardiac responses were observed and their evolution during training seem to reflect both associative and non-associative processes. The results are interpreted as an evidence of differential rate of maturation of the CNS structures involved in associative processes underlying emotional (preparatory) and leg flexion (consummatory) responses elicited by noxious stimuli.

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758 ELEVATION OF BRAIN TYROSINE CONCENTRATIONS BY CYCLOHEXIMIDE IS NOT RESPONSIBLE FOR ITS AMNESIC EFFECTS. Curt W. Spanis and Larry R. Squire. Psychology Service, V.A. Hospital, and Psychiatry Dept., UCSD, La Jolla, CA 92161.

Administration of cycloheximide, an inhibitor of protein synthesis, elevates brain tyrosine concentrations. This finding raised the possibility that the amnesic effect of this drug could be due to abnormal brain tyrosine concentrations. We have found that 1) amnesic doses of cycloheximide produced a 200-250% elevation in brain tyrosine concentrations that persisted for several hours after injection; 2) injection of L-tyrosine, in doses that elevated brain tyrosine more than cycloheximide, did not affect memory. Therefore, the effect of protein synthesis inhibitors on brain tyrosine is not sufficient to explain their amnesic effect.

759 ANTEROGRADE AMNESIA FOLLOWING ECT: AN ANALYSIS OF THE BENEFICIAL EFFECTS OF PARTIAL INFORMATION. Larry R. Squire, C. Douglas Wetzel*, and Pamela C. Slater*. Psychology Service, V.A. Hospital, and Psychiatry Dept., UCSD, La Jolla, CA 92161.

Patients receiving bilateral ECT learned a list of words and then were tested for retention by three different retention procedures -- partial information, yes-no recognition, and 2-choice recognition. A comparison of all three retention conditions indicated that the yes-no recognition condition was particularly difficult for amnesic patients. Moreover, at short learning-retention intervals, the partial information procedure was beneficial for amnesic patients but not for normal subjects. These results confirmed previous findings with the partial information procedure and suggested that the amnesic syndrome can exhibit qualitatively distinct features not apparent in normal memory. During the course of forgetting, however, normal subjects came to exhibit a nearly identical pattern of performance as amnesic patients. These findings indicated that apparently qualitative defects in performance can sometimes be characterized as a quantitative change along a normal continuum. It seems possible that there are important similarities between the poor memory of amnesic patients and the poor memory of normal subjects long after learning.

760 PHASE-SHIFTS OF CIRCADIAN RHYTHMS PRODUCE RETROGRADE AMNESIA. Walter N. Tapp* and Frank A. Holloway. Dept. Psychiatry and Behav. Sci., The University of Okla. Health Sci. Center, Okla. City, Ok. 73190.

Male albino rats were entrained to a 12:12 light-dark (LD) cycle. Rats were housed in photocell activity cages and activity was recorded throughout the experiment. Rats were trained in a one-trial passive avoidance task. Shortly after training, the LD cycle was phase-shifted 0, 6, or 12 h. Testing was 48 h or 7 days after training.

Training and testing did not affect the circadian activity rhythms of control rats which were not phase-shifted. These animals showed stable, high levels of retention performance at 48 h and 7 days. Following LD shifts, the circadian activity rhythms showed temporary disturbances characteristic of resynchronization to a new LD cycle. Disturbed circadian activity rhythms usually appeared within 24 h of the phase-shift and disappeared within 5 days. Retention performance of phase-shifted animals was impaired 48 h or 7 days after training. Since retention deficits were seen in phase-shifted animals trained and tested in the same light condition, explanations based on generalization decrements due to differences in light conditions can be excluded. Similarly, retention impairments 7 days after training suggest that the deficit is not due to impaired performance since the animals had resynchronized to the new LD schedule by then. Two more plausible explanations invoke storage or retrieval mechanisms. Disruption of the animal's internal rhythmic organization may be sufficient to disrupt memory storage. Phase-shifting internal rhythms may alter the animal's internal state leading to a state-dependent retrieval deficit.

761 FACILITATORY EFFECTS OF RESERPINE IN AN INHIBITORY AVOIDANCE TASK. Beatriz J. Vasquez*, Joe L. Martinez, Jr., Robert A. Jensen, and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717 U.S.A.

Reserpine is a monoamine depletor that interferes with storage mechanisms. Some studies report that reserpine has no effect on memory while other research indicates that it can produce amnesia in both active and inhibitory avoidance tasks. Part of the contradictory findings may be related to different levels of footshock employed and to circadian differences arising from the use of variable treatment-test intervals. This study was designed to address itself to these problems. Two footshock levels (350 uA, 2 mA) and two doses of reserpine (0.3 and 3.0 mg/kg) given i.p. either 6 hr before training, immediately after or 6 hr after training, were used in this experiment. Circadian differences were assessed by comparing each treated group to a control that had the same training-treatment interval. Male Swiss-Webster mice were trained in an inhibitory avoidance task using a two-chamber straight alley apparatus. Animals were placed into a well-lit start compartment separated from a dark and larger shock chamber by a guillotine door. When the mouse entered the dark compartment it received a footshock that terminated when the animal escaped back to the start compartment. Retention tests were given 72 hr later; latencies to step-through were recorded to a maximum of 600 sec.

In the groups that received a 350 uA footshock 6 hr before training, 3.0 mg/kg reserpine resulted in impaired retention performance ($U=671$; $Z=2.57$; $p=.01$). However, when reserpine was given 6 hr after training (3.0 mg/kg; 350 uA) a significant facilitation of retention performance was observed ($U=346$; $Z=2.48$; $p=.0132$). In the 2 mA footshock condition, 3.0 mg/kg reserpine given 6 hr before training produced an impairment of retention ($U=513$; $Z=2.57$; $p=.01$). When the same treatment (3.0 mg/kg; 2 mA) was administered immediately after training it produced a significant facilitation of retention performance ($U=228$; $Z=1.89$; $p=.0588$).

The fact that reserpine facilitated retention performance when given after the training trial indicates that the drug was acting to enhance some aspect of the memory consolidation process and not influencing performance factors, although the latter cannot be completely ruled out. Impairment of retention was seen only with the high dose of reserpine injected 6 hr before training. Animals under this condition were behaviorally depressed at the time of training as evidenced by their longer initial entrance latencies. Therefore, with pre-training injections, the effects on retention might be due to interference with acquisition rather than or in addition to storage processes. (Supported by UPHS grants MH 05249, MH 05358, MH 12526.)

762 DOPAMINERGIC BASIS OF SELF-STIMULATION INDUCED IMPROVEMENT IN MEMORY. Norman White and Robert Major*. Dept. Psychol., McGill Univ., 1205 McGregor Ave., Montreal, Canada.

Naive, thirsty rats searched for a standard drinking tube which was located in one wall of an open field. For one group of rats the tube contained water, for another group the tube was dry. As each rat contacted the tube it was removed from the open field and placed into a self-stimulation cage. One half of the rats in the water group, and one half of the rats in the no-water group were allowed to bar press 1000 times for lateral hypothalamic stimulation. The remaining rats in each group were simply confined in the self-stimulation cage for 30 min. Twenty-four hours later each rat was put into the open field and the latency to contact the drinking tube (which was dry for all groups) was recorded. The rats which found water in the drinking tube on the training day and self-stimulated afterwards had significantly shorter latencies than the rats which found water but did not self-stimulate. The rats which found a dry drinking tube on the training day and self-stimulated afterward had significantly longer latencies to contact the tube (most did not approach it in 5 min) than the rats which found a dry tube and did not self-stimulate. We interpreted this finding as indicating that self-stimulation improved the rats' memory for prior events even though the stimulation was not a part of the contingent association that the rats learned to produce the observed performance. The data from three additional experiments using the same experimental paradigm suggest that the dopaminergic nigrostriatal bundle mediates the memory effect that we observed. 1) Electrode placements in the far lateral hypothalamus produced the memory effect, but electrode placements in the mid-lateral hypothalamus did not, even though both placements produced equal rates of bar pressing. 2) Electrode placements in area A9 produced the memory effect, but electrode placements in the pre-optic area did not, even though both placements produced equal rates of bar pressing. 3) A dose of 0.3 mg/Kg of the dopaminergic blocker Pimozide, given 4½ hrs before testing, eliminated the memory effect in rats with far-lateral placements without affecting rates of self-stimulation. The appropriate vehicle control injections had no effect on rates of self-stimulation or on the improvement of memory. As we did not observe any reduction in rate following our dose of Pimozide the data suggest that the effects of specific dopaminergic manipulations on self-stimulation may be interpretable in terms of an effect on memory similar to the one reported here: this effect could have occurred inadvertently in other experiments. The data also confirm recent suggestions that the nigro-striatal bundle is involved in the consolidation of memory for some kinds of learning, and demonstrates that memory can be improved by self-stimulation; that is, by stimulation that is clearly reinforcing.

MONOAMINERGIC SYSTEMS

763 LOCUS COERULEUS LESIONS: INCREASE IN NOCICEPTIVE THRESHOLDS. Robert F. Ackermann*, Richard J. Bodnar, Dennis D. Kelly, and Murray Glusman. New York State Psychiatric Institute and Albert Einstein College of Medicine, New York, N.Y.

Serotonin (5-HT) depletion or lesions of the dorsal raphé nuclei of the brainstem have been shown to attenuate morphine-produced and stimulation-produced analgesia. In contrast, norepinephrine (NE) depletion enhances morphine-produced and stimulation-produced analgesia. The present study examined the effects of destruction of the locus coeruleus (LC), a noradrenergic pontine nucleus, upon nociceptive flinch-jump thresholds. Thirteen rats were tested for four preoperative baseline flinch-jump sessions. Lesions were then placed in the locus coeruleus bilaterally and nociceptive thresholds were determined daily for 5 weeks post-operatively. The lesions were localized by catecholamine histofluorescence procedures as well as by conventional histological staining techniques. In 9 of the 13 animals, either bilateral or unilateral damage was found in the LC or in the ascending dorsal noradrenergic bundle, as evidenced by green fluorescent back-up caudal to the lesions. Eight of the nine animals in this group demonstrated significantly increased jump thresholds. In the remaining four animals, both lesions spared the LC but caused damage of serotonergic elements in the raphé region, as evidenced by yellow fluorescent back-up caudal to the lesions. These raphe-damaged animals demonstrated either unchanged or significantly decreased jump thresholds. The results suggest apparently contrasting roles of NE and 5-HT in nociception. (Supported by NIMH Grant #13579, New York State Health Research Council Grant #365 and by Grant #NS 09649.)

764 AN INVESTIGATION OF THE ROLE OF THE MEDIAL NUCLEUS OF THE RAPHE IN BEHAVIOR. Karen E. Asin, David Wirtshafter and Ernest W. Kent. Dept. Psych, Univ. Il. at Chicago Circle, Chicago, Il. 60680

Last year we reported that lesion of the medial but not the dorsal nucleus of the raphe impaired both the acquisition and extinction of a food rewarded runway task (Neurosci. Abst. II, #676). In an elaboration of our initial study, we have evaluated the effect of the intertrial interval on the acquisition of a straight alley task. Medial raphe lesioned (MRL) rats were deficient relative to controls in acquiring the response when the trials were spaced 15 minutes apart but not when the trials were immediately successive. This result may suggest that the acquisition deficit is due, in part, to distraction of MRL animals by stimuli encountered between trials. As a measure of reactivity to changes in stimuli within the alley, MRL and control subjects which had previously been trained were run for six trials during which the floor of the alley was covered with sandpaper. Control rats demonstrated a marked initial decline in running speeds, with a rapid recovery to baseline levels. MRL rats, however, showed a less pronounced diminution in speed, but were slower than controls to return to baseline.

Subsequently, spontaneous alternation was studied over two successive, non-rewarded trials in a T-maze. Compared to control animals, MRL rats displayed strong perseverative tendencies, confirming the findings of Geyer et al. (Br.Res.106,1976). We extended these findings to methysergide and p-chlorophenylalanine (PCPA) treated subjects, who also tended to repeat their initial choice. When the two arms of the maze were reversed between trials, behavior on the second trial was essentially random. Therefore, it appears that the nature of the repetitive responding cannot be described solely in terms of either stimulus or response perseveration. Additionally, we found that 5-HTP treatment of PCPA injected animals was able to restore spontaneous alternation to control levels. Perseverative responding was also investigated in a straight alley task in which alternate trials were food rewarded. Whereas control rats eventually ran faster on rewarded than non-rewarded trials, MRL rats failed to respond differentially.

The above results indicate that there are both similarities and differences in the behavioral consequences of medial raphe and hippocampal or septal lesions. The extent to which serotonin reduction in these limbic structures underlies the behavioral effects seen in MRL subjects remains to be determined.

765 ASCENDING SEROTONERGIC SYSTEMS CONTROLLING RHYTHMICAL ACTIVITY IN THE SEPTAL AREA OF THE RAT. S.Y. Assaf* and J.J. Miller, Dept. Physiology, Univ. British Columbia, Vancouver, B.C.

Stimulation of hypothalamic and brainstem regions have been shown to elicit contrasting patterns of hippocampal electrical activity (HEA). These responses, either rhythmical slow wave 'theta' or a desynchronized pattern are correlated with a bursting or irregular firing of neurones in the medial septal nucleus respectively. Recent neuroanatomical investigations have demonstrated that projection systems originating in the monoamine-containing nuclei of the brainstem may form the neural substrates through which these distinct patterns of activity in the hippocampus and septum are altered. The present study was undertaken to determine the effects of stimulation of the ascending serotonergic system on the extracellularly recorded unit activity in the septal area and to relate these responses to HEA. On the basis of their discharge patterns, two types of medial septal neurones were identified; (1) I-neurones, which characteristically fired in an irregular or random pattern and exhibited no temporal relationship with HEA and (2) B-neurones, which discharged either in rhythmical bursts (2-10 spikes) correlated with the 'theta' response or in an irregular manner during desynchronized HEA. Single pulse stimulation (10-15V) of the median raphe nucleus (MR) inhibited the discharge of I-neurones for periods of 30-200 msec but did not influence B-neurones. Repetitive stimulation of the MR at low intensities (2-3V, 100 Hz) prolonged the inhibition of I-neurones and resulted in the disruption of the bursting discharge of B-neurones as well as a shift of HEA from the 'theta' mode to a desynchronized pattern. Depletion of central serotonin levels (85-93%) following injections of p-CPA (400 mg/kg) 4 days prior to acute recordings resulted in a blockade of the MR elicited responses in both the septum and hippocampus. These data suggest that I-neurones are monosynaptically inhibited by a serotonergic projection from the MR and that these cells in turn mediate the disruption of the bursting discharge of B-neurones resulting in the desynchronization of HEA.
(Supported by the Medical Research Council of Canada.)

766 PLASMA AND BRAIN LEVELS OF HOMOVANILLIC ACID (HVA) AND 3,4,-DIHYDROXYPHENYLACETIC ACID (DOPAC): EFFECTS OF HALOPERIDOL AND NIGROSTRIATAL PATHWAY STIMULATION. N. G. Bacopoulos*, S. E. Hattox*, R. H. Roth and J. W. Maas. Departments of Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510.

The concentrations of HVA and DOPAC were measured in rat caudate nucleus and plasma by GC-mass fragmentography. Blood (5-6 ml) was collected by cardiac puncture and centrifuged at 10,000 x g for 20 min. Deuterated HVA and/or DOPAC were added to plasma aliquots or supernates of caudate nucleus as internal standards. Catechol acids were extracted into ethyl acetate from acidified plasma aliquots or supernates of caudate nucleus homogenates. The ethyl acetate was evaporated and the pentafluoropropionyl derivatives of HVA or DOPAC were formed by reaction with pentafluoroproprionic acid anhydride and pentafluoropropanol. Quantitation was performed by the technique of selected ion monitoring using a Finnigan model 3200 quadrupole electron impact mass spectrometer. Ions at m/e 460 and 462, or 387 and 392 originating from endogenous and deuterated HVA and DOPAC respectively were monitored. Identification was verified by comparing ratios of two ions derived from each endogenous compound. The level of free HVA in rat plasma was 4.5 ± 0.321 ng/ml (N=8, x ± S.E.M.). The level of plasma HVA was increased 50 ± 5.6% two hours after administration of haloperidol (1.25 mg/kg s.c.). Further increases in the dose of haloperidol did not cause any additional elevations in plasma HVA. Stimulation of the nigrostriatal pathway for 30 minutes at a frequency of 15 Hz (Murrin, L.C. and Roth, R.H., Mol. Pharm. 12:463, 1976) resulted in a significant increase in the levels of HVA (73 ± 12%) and DOPAC (153 ± 38%) in the caudates ipsilateral to the stimulation. The HVA concentration in the plasma of stimulated animals was increased 22.5 ± 6.2% ($p < 0.05$) as compared to unstimulated controls. These experiments suggest that changes in the concentration of HVA in the plasma may reflect changes in the activity of central dopamine neurons. Experiments are in progress to determine the relative contribution of brain to plasma levels of dopamine metabolites.

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767 VERATRAMINE: EVIDENCE FOR CENTRAL SEROTONINERGIC AGONIST ACTION. Roger F. Butterworth, Kanji Izumi*, Michel Gonce* and André Barbeau. Dept. of Neurobiology, Clinical Research Institute of Montreal, Montreal, Quebec, Canada.

The secondary amine, Veratramine, was first isolated by Saito (Bull. Chem. Soc. Japan, 15, 22 (1940)) from the Japanese Veratrum Grandiflorum, followed later by others from the American Veratrum Viride. The most noteworthy action of Veratramine is its excitatory action on the central nervous system; doses in excess of 1.4 mg. per kg. administered i.p. to male Swiss albino mice produce tremor within a few minutes of administration. At a dose of 3 mg. per kg., the excitatory pattern consists of a more intense tremor followed by a characteristic "struggling", and in many cases, convulsive behaviour.

The behavioural excitation produced by Veratramine is accompanied by changes in serotonin (5HT) content of certain brain regions while brain dopamine (DA) and noradrenaline (NA) remain unchanged. The following drugs, in the doses indicated were found to be without effect on the excitatory behaviour produced by Veratramine (3 mg. per kg., ED_{99}): Haloperidol 5 mg. per kg.; Phentolamine 10 mg. per kg.; Propranolol 10 mg. per kg.; Atropine 10 mg. per kg.; α-methyl-p-tyrosine 250 mg. per kg.; p-chlorophenylalanine 300 mg. per kg. Previously Tanaka (J. Pharmacol. Exp. Ther. 113, 89 (1955)) showed that the anticonvulsants phenobarbital and diphenylhydantoin were only partially effective as inhibitors of Veratramine's action and this, only at toxic doses.

The central serotonin receptor antagonist, methysergide in doses of 5-15 mg. per kg. produced a dose-dependent inhibition of Veratramine's action, causing both a decreased intensity of excitation and a delay in onset of tremor. These results suggest a serotonin agonist role for the mechanism of excitatory action of Veratramine on the central nervous system.

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768 EFFECTS OF UNILATERAL FOREBRAIN DEPLETIONS OF SEROTONIN, NOREPINEPHRINE AND DOPAMINE ON HYPOTHALAMIC SELF-STIMULATION. Robert J. Carey. VAH at Syracuse, Syracuse, NY 13210.

Rats with functional bilateral medial forebrain bundle electrodes were administered 2-3 unilateral 2 µl injections of 6-hydroxydopamine (2 µg/µl) into the substantia nigra over a two week period. The injections which produced 99% depletion of striatal-cortical dopamine either had no effect on self-stimulation rate intensity functions or produced equivalent bilateral decreases in self-stimulation. Instances of decreases in self-stimulation were always associated with marked decreases in motor activity as indicated by photobeam activity measurement. Thus, virtually complete unilateral forebrain dopamine depletions had no selective effect on hypothalamic self-stimulation. Additional rats, however, were given unilateral 6-hydroxydopamine injections into the dorsal and ventral norepinephrine pathways in combination with 6-hydroxydopamine injections into the substantia nigra. The threshold for self-stimulation in these rats was markedly lowered on the injected side. Thus, unilateral forebrain depletions of dopamine (99%) plus norepinephrine (90-95%) selectively enhanced hypothalamic self-stimulation. Finally, if a lesion of the midline raphe nuclei was made in combination with a unilateral 6-hydroxydopamine injection into the substantia nigra self-stimulation was attenuated on the injected side. These studies suggest that serotonin and norepinephrine but not dopamine are important modulators of hypothalamic self-stimulation.

769 ACTIVATION OF LOCUS COERULEUS NORADRENERGIC NEURONS BY PERIPHERAL NERVE STIMULATION. J.M. Cedarbaum* and G.K. Aghajanian (Spon: M. Davis), Depts. Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508

Previous experiments have shown that, even in anesthetized animals, the noradrenergic neurons of the locus coeruleus (LC) respond to noxious stimuli with a brief increase in firing rate followed by a quiescent interval, after which the cells resume their normal pattern of activity. Experiments in which horseracish peroxidase was injected into the LC revealed that the LC receives direct, monosynaptic input from large, presumably nociceptive neurons located in the marginal zones (Rexed Lamina I) of the dorsal horns of the spinal cord. To further study the response of LC neurons to peripheral stimuli, we have stimulated peripheral nerves (saphenous, sural or sciatic) in the hind lege of chloral hydrate-anesthetized rats while recording single unit activity in the LC. The response of LC cells to nerve stimulation (single square-wave pulses, 0.05-5 mA, 1 msec duration, at a rate of 0.5 Hz) consisted of short and long latency components. At low stimulus intensities (0.05-1 mA), LC cells responded with a single spike/stimulus (latency 18-35 msec). Higher stimulus intensities (up to 5 mA) evoked bursts of several additional spikes/stimulus, beginning 50-100 msec following the initial spike. Stimuli delivered contralateral to the recording site in the LC were more effective than ipsilateral stimuli, the threshold for evoking a response in the LC being 5-10 fold higher for ipsilateral than for contralateral stimulation. The evoked activity of LC units was prevented by lesioning the anterolateral quadrant of the spinal cord contralateral to the stimulated nerve and ipsilateral to the recording site in the LC. Lesions of the dorsal columns were without effect. The evoked spike activity of LC cells was followed by a period of suppressed cell firing lasting in some cases up to a full second. Early during this period of suppressed activity the response to the second of a pair of stimuli was markedly diminished. The duration of this suppression was reduced by the α-adrenergic blocking drug piperoxane, administered either systemically or directly into the vicinity of LC cells by microiontophoresis. This suggests that the post-stimulus suppression of firing is adrenergically mediated, perhaps by collaterals of the LC cells themselves. Thus the LC appears to receive input from the spinal cord, most likely from branches of nociceptive spinoreticular and/or spinothalamic fibers traveling in the anterolateral white matter. The response of LC cells to peripheral stimuli is powerfully gated, presumably by α-adrenoreceptor-mediated collateral inhibition within the LC. Such a mechanism might allow the LC to function as a "novelty detector" responding acutely and transiently to external events. (USPHS Grant MH-17871 and the State of Connecticut).

770 THE EFFECTS OF ORTHODROMIC AND ANTIDROMIC STIMULATION ON TYROSINE HYDROXYLASE ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION. A. Chalazonitis* and R.E. Zigmond (SPON: P.B. Dews). Department of Pharmacology, Harvard Medical School, Boston, MA 02115

Stimulation of the preganglionic cervical sympathetic trunk produces a delayed increase in tyrosine hydroxylase activity in the rat superior cervical ganglion (Ben-Ari and Zigmond, J. Physiol. 248: 48P, 1975). To determine whether stimulation of nicotinic receptors in the ganglion is involved in this effect, rats were pretreated with the ganglion blocking drug, chlorisondamine (15 mg/kg, s.c.). Sixty minutes later the animals were anesthetized with chloral hydrate (440 mg/kg) and the preganglionic trunks were exposed and cut bilaterally. The distal portion of the trunk on one side was then stimulated for 90 min with 40 Hz trains (current on for 250 msec, off for 500 msec). We have previously shown that these parameters of stimulation produce approximately a two-fold increase in tyrosine hydroxylase activity in the ganglion. Electrophysiological recordings throughout the period of stimulation demonstrated that the chlorisondamine treatment completely blocked the evoked potentials normally recorded from the surface of the ganglion. Subsequent measurement of tyrosine hydroxylase activity showed that the nicotinic antagonist significantly decreased the effect of preganglionic stimulation on this enzyme activity. Seventy-two hours after the stimulation, ganglionic tyrosine hydroxylase activity was 85% higher on the stimulated side than on the contralateral control side of uninjected animals but only 24% higher in chlorisondamine pretreated animals.

In order to determine whether increased neuronal firing of the post-ganglionic neurons per se (i.e., without release of the preganglionic transmitter) was sufficient to produce an elevation of tyrosine hydroxylase activity, the ganglion on one side was stimulated antidromically via the internal carotid nerve. The postganglionic trunks were not cut in this experiment. The antidromically evoked compound action potential was recorded and did not diminish significantly during the period of stimulation. Although several parameters of stimulation were tried, none produced an elevation in tyrosine hydroxylase activity compared to "sham stimulated" controls (i.e. animals treated identically except the current was not turned on). The results suggest that the elevation in tyrosine hydroxylase activity produced by synaptic stimulation is not simply due to an increase in the frequency of action potentials in sympathetic neurons.

We are currently trying to determine the minimum amount of preganglionic stimulation required to produce a significant increase in tyrosine hydroxylase activity and have found that a 10 min period of stimulation at 10 Hz is sufficient to produce a small (25%) but significant rise in enzyme activity.

771 DEMONSTRATION OF INDOLEAMINE NEURONS AND THEIR PROCESSES IN THE RAT BRAIN; SELECTIVE LOSS IN THIAMINE DEFICIENCY AND REGENERATIVE PLASTICITY WITH PYRITHIAMINE INDUCTION AND THIAMINE READMINISTRATION. Victoria Chan-Palay. Dept. Neurobiol., Harvard Med. Sch. Boston, MA 02115.

Indoleamine structures in normal, pair-fed control and thiamine-deficient brain of rats are selectively localized by autoradiography following serotonin oxidase inhibition and simultaneous administration of ^{3}H-5HT (10^{-5}M) and cold norepinephrine (10^{-4}M) by continuous intraventricular infusions of 3 hr duration. Locations of indoleamine neurons in the normal and control brains are comparable in the brain stem, diencephalon, epithalamus and circumventricular organs. These emit vast axonal plexuses throughout the brain, ventricular, and leptomeningeal surfaces. Thiamine deficiency causes a dramatic loss of almost all indoleamine neurons in midbrain and medulla, in which rare dystrophic cells and axons survive. In the diencephalon a few neurons survive with commensurately greater axon preservation. The periventricular grey of spinal cord, medulla, midbrain and diencephalon; mammillary nuclei, cerebellum and floor of IVth ventricle, normally richly served by indoleamine axons, are the areas most severely affected. However, the ventricular and leptomeningeal plexuses remain intact. It is postulated that thiamine deficiency may cause a change in the uptake of exogenous 5HT and destruction of certain indole-containing structures. Of these latter, the synaptic indoleamine systems such as the cerebellar 5HT mossy fibers which synapse within glomeruli disappear. The nonsynapsing indoleamine systems that utilize neurohumoral transmitter dispersion instead of localized synapses persist, e.g., the ventricular axons. Chronic (4-6 wks) diet-induced thiamine deficiency is not readily reversible. Acute treatment with pyrithiamine (thiamine antagonist, 20µgm/day for 4-6 days) causes rapid indoleamine loss. However, upon readministration of thiamine (20µgm/day for 2-14 days) indoleamine-containing structures rapidly regenerate. These experiments demonstrate the intimate dependence of the indoleamine systems of the brain upon thiamine, destruction of these structures in the vitamin deficient animals and their regenerative plasticity upon readministration of thiamine. The distribution of greatest indoleamine deficit in the thiamine deficient brain coincides with those regions showing pathologic changes in Wernicke-Korsakoff's syndrome in man: the mammillary bodies, medial thalamus and hypothalamus, periventricular grey, floor of IVth ventricle and cerebellum. This indoleamine loss constitutes a pathognomonic change of thiamine deficiency, though not necessarily the only one. These experiments are excellent models for study of mechanisms involved in the human disease. (Supported in part by PHS Grants NS 10536, NS 03659 and Training Grant NS 05591 from the NINCDS.)

772 EFFECTS OF LSD ON THE ACTIVITY OF DOPAMINE-CONTAINING NEURONS IN THE SUBSTANTIA NIGRA OF RATS. Greg R. Christoph*, Donald H. Kuhn and Barry L. Jacobs. Dept. Psychol., Princeton Univ., Princeton, N.J. 08540.

Behavioral and neurochemical evidence suggests that d-lysergic acid diethylamide (LSD) directly interacts with dopamine (DA) receptors in the CNS (Pieri, et al., Nature 252: 586, 1974; Creese, et al., Life Sci. 17: 1715, 1975). In order to more directly investigate the dopaminergic properties of LSD, its effects on the spontaneous firing rate of DA-containing neurons in the substantia nigra were studied. In chloral hydrate anesthetized rats, DA-containing neurons were identified on line by their firing rate (2-7 spikes/sec) and spike duration (> 2 msec) characteristics. DA agonists are known to reduce the firing rate of these neurons, and DA antagonists block or reverse this effect. Low doses of LSD (25-50 ug/kg, i.v.) significantly depressed the firing rate of 77% of the DA containing neurons in the zona compacta of the substantia nigra, whereas 23% of the cells were unaffected or slightly excited. Pretreatment with the DA antagonist haloperidol (0.1 mg/kg, i.v.) blocked the inhibitory effects of LSD, and haloperidol injected after LSD reversed its depressive effects. Suppression of firing rate was probably not due to the serotonergic properties of LSD, since 5-methoxy N,N dimethyltryptamine (25-100 ug/kg., i.v.), a drug that has serotonergic properties similar to those of LSD, exclusively produced excitation. Non-dopaminergic cells in the region of the substantia nigra were typically greatly excited by administration of LSD. These results indicate that LSD can act as a DA agonist in the CNS. Dopaminergic cells were also tested with LSD (50 ug/kg) after the firing rate was reduced 50% by pretreatment with d-amphetamine (AMPH, mean dose = 1.2 mg/kg, i.v.). LSD increased the firing rate of most of these cells to a level slightly below the pre-AMPH baseline. Dopaminergic cells that were tested with LSD (200 ug/kg) and allowed to recover, were then tested with AMPH. The 50% inhibition dose of AMPH (4.7 mg/kg) was significantly greater for these rats than for rats without prior LSD. The results of these AMPH/LSD interaction experiments may indicate that LSD also has DA antagonist properties. These electrophysiological data are consistent with neurochemical data which suggest that LSD is a mixed agonist/antagonist of DA in the CNS.

773 MAPPING OF BRAIN STIMULATION REWARD: PONTINE TEGMENTUM AND CAUDAL MIDBRAIN. Dale Corbett* and Roy A. Wise. (Spon: J. Stewart). Dept. Psychology, Concordia Univ., Montreal, Canada H3G 1M8.

Recent lesion studies cast doubt on the view that noradrenergic (NA) systems mediate pontine tegmental and caudal midbrain self-stimulation (SS). It is not apparent, however, what alternative systems might underlie SS in these areas. In order to uncover candidate systems, 411 stimulation sites were tested in 46 rats using chronic moveable electrodes (Wise, 1976). Both positive and negative SS electrode placements were examined in nissl-stained tissue as well as in glyoxylic acid treated tissue for the demonstration of catecholamines.

Self-stimulation was obtained from the following areas: dorsal raphe nucleus (RD); ventral periaqueductal gray; superior cerebellar peduncle (PCS); pontine tegmental gray; mesencephalic nucleus of the trigeminal (MesV); and motor nucleus of the trigeminal (MotV). Electrode placements on the lateral border of the locus coeruleus (LC) adjacent to MesV supported SS, while those on the medial edge of LC failed to support SS despite extensive behavioral shaping. This supports the view (Vanderkooy and Phillips, 1977) that MesV is the critical site for SS with electrodes in the area of LC. SS was closely correlated with the rostral (caudal midbrain) and caudal (posterior LC) cells of MesV. While NA cell bodies or fibers were evident in all regions that supported SS, the location of positive SS placements often did not coincide with the areas most densely innervated by NE elements. For example, SS was readily obtained from RD, where there were relatively few NE fibers, while SS was not obtained dorso-lateral to RD where abundant NA fibers were evident. A similar pattern was observed with placements in the PCS and the pontine tegmental gray.

These data suggest that NA systems in the pontine tegmentum and caudal midbrain do not support self-stimulation. Rather, it seems that the trigeminal system plays an important role in brain stimulation reward in these regions. This finding has important implications for current theories of motivated behavior.

774 LOCUS COERULEUS STIMULATION INCREASES PLASMA LEVELS OF 3-METHOXY-4-HYDROXYPHENYLETHYLENEGLYCOL (MHPG) IN RATS. J. N. Crawley, J. W. Maas, R. H. Roth and S. E. Hattox* Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510

3-Methoxy-4-hydroxyphenylethyleneglycol (MHPG) is a major metabolite of norepinephrine (NE) formed from both central and peripheral adrenergic neurons. To determine whether the functional activity of central noradrenergic neurons is reflected in plasma levels of MHPG, we have electrically stimulated a homogeneous group of central NE neurons, the locus coeruleus (LC) in rats.

The LC was stereotaxically and electrophysiologically located in urethane-anesthetized rats, with a tungsten bipolar electrode (external diameter 0.5 mm, internal fine wire diameter 0.1 mm). Stimulation through this electrode was bipolar, with a peak-to-peak amplitude of 400 µA, duration 2 msec, at 20 Hz, for 30 minutes. Control (sham operated) rats were identically prepared but stimulation current was not passed. Immediately thereafter, 4-6 ml blood was removed by cardiac puncture for enzymatic hydrolysis and ethyl acetate extraction of total MHPG, and quantification by gas chromatography-mass spectrometry. To verify effective LC stimulation, the cerebral cortex hemispheres ipsilateral and contralateral to LC stimulation were dissected and frozen at -70°C for gas chromatographic assay of MHPG. In LC stimulated rats, total MHPG increased 30-80% in the ipsilateral cortex as compared to contralateral, while the two sides were similar in sham controls. The rest of the brain was stored in formalin for histological sectioning and staining with cresyl violet to verify correct electrode placement in the locus coeruleus.

Plasma levels of total MHPG in sham controls averaged 10.6 ng MHPG/ml plasma (±1.41 SEM, N=4). Total plasma MHPG in locus coeruleus stimulated rats averaged 30.1 ng/ml (±3.16 SEM, N=6), $p<0.01$. Experiments with ganglionic blocking agents demonstrate that a significant portion of the plasma MHPG increase during LC stimulation is derived from the central nervous system. Further experiments are in progress to dissect out the central vs. peripheral contributions to plasma MHPG. The results herein indicate that plasma MHPG does reflect changes in functional activity of central NE neurons.

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775 STUDIES ON THE POSSIBILITY OF AN EXTRAGANGLIONIC SOURCE OF ADRENERGIC TERMINALS TO THE SUPERIOR CERVICAL GANGLION. W.G. Dail and Joe Wood. Dept. Anat., Univ. New Mexico Sch. Med., Albuquerque, NM 87131, and Dept. Neurobiol. and Anat., Univ. Texas Med. Sch., Houston, TX 77025.

In the sympathetic ganglia of many mammals, adrenergic varicose fibers are found in close proximity to ganglion cells. In the superior cervical ganglion (SCG), it has been suggested that such fibers represent the source of catecholamines released by stimulation of the cervical sympathetic trunk. The varicose fibers are said to arise predominantly from small intensely fluorescent cells or from ganglion cells within the SCG. That a portion of these fibers may arise from adrenergic ganglion cells caudal to the SCG is a recent and alternate proposal. To test this latter hypothesis, we have performed histofluorescence and EM studies of the SCG of the rabbit. Ligatures placed on the cervical sympathetic trunk resulted in the accumulation of catecholamines in fibers proximal to the tie, indicating a flow of the neurotransmitter toward the SCG. Moreover, following section of the cervical sympathetic trunk, chromatolytic changes were observed in some of the ganglion cells caudal to the lesion. When the normal SCG was fixed with $KMnO_4$ it was revealed that approximately 25% of the synaptic profiles were of the adrenergic type. Two days following deafferentation of the SCG, cholinergic terminals could no longer be identified. However, adrenergic terminals were commonly seen. Counts of the adrenergic profiles and degenerating terminals indicated that the proportion of adrenergic terminals remained at 25% following section of the cervical sympathetic trunk.

These data indicate that the superior cervical ganglion is not a target organ for adrenergic fibers found in the cervical sympathetic trunk.

776 SENSITIZATION OF POSTSYNAPTIC SEROTONIN RECEPTORS BY CHRONIC PRETREATMENT WITH TRICYCLIC ANTIDEPRESSANTS: AN IONTOPHORETIC STUDY. C. de Montigny and G.K. Aghajanian, Depts. Psychiat. & Pharmacol. Yale Univ. Sch. Med., New Haven, CT 06508

The monoamine hypothesis of depression is partly based on the therapeutic efficacy of tricyclic compounds which block amine uptake. However there is a time discrepancy between the acute pharmacological and the delayed clinical effects. Furthermore, no clear difference has been demonstrated between the clinical efficacies of norepinephrine (NE) and serotonin (5HT) uptake blockers.

To approach these questions, we studied the acute and chronic effects on postsynaptic 5HT receptors of two antidepressants: chlorimipramine (CIMI), a 5HT uptake blocker, and desipramine (DMI), a NE uptake blocker. Male albino rats were pretreated for 1-17 days with a daily dose of 5 mg/kg, i.p., of CIMI or DMI, the last dose being given 24 h before testing. Recordings were also obtained 15-90 min following 5 mg/kg, i.p., of the same drugs. In a cerveau isole preparation unit recordings were obtained from the ventral lateral geniculate body (LGB), which receives a dense 5HT input. 5HT was pulsed iontophoretically for 60 sec onto these neurons in alternation with γ-aminobutyric acid (GABA). The efficacy of these compounds was evaluated by the IT_{50} values; i.e., the product of the current I by the time T_{50} required to obtain a 50% depression from the baseline firing rate.

Acute administration of CIMI and DMI did not modify the initial response (i.e., sensitivity) of LGB neurons to 5HT or GABA. CIMI, but not DMI, prolonged the recovery time from the depression induced by 5HT. Recordings obtained 24 h after a single dose of CIMI or DMI failed to disclose any significant change of the IT_{50} values for both 5HT and GABA. However, in animals pretreated chronically with CIMI, LGB neurons were found to be much more sensitive to 5HT but not GABA. Chronic DMI pretreatment also enhanced selectively the sensitivity to 5HT. IT_{50} measures revealed an increase sensitivity to 5HT of 2 to 4 fold.

These results suggest that chronic administration of both types of antidepressants sensitizes postsynaptic 5HT receptors. The enhanced response of LGB neurons to 5HT cannot be due to the block of 5HT uptake since acute CIMI failed to modify sensitivity. The progressive sensitization and its long duration contrasts with the immediate and short lasting presynaptic effects of these drugs on amine uptake. Moreover, despite dissimilarities in their amine uptake blocking properties, CIMI and DMI exert a very similar action on postsynaptic 5HT receptors. Thus the common action of these tricyclic drugs in producing a delayed sensitization of postsynaptic 5HT receptors correlates better than their presynaptic effects with therapeutic efficacy in depression. (Supported by NIMH Grants MH-17871 and MH-14459 and Can. Med. Res. Council).

777 THE STAGES OF BEHAVIOR PRODUCED BY CONTINUOUS AMPHETAMINE INTOXICATION ARE ALTERED BY LESIONS OF CATECHOLAMINE CELLS. Michael S. Eison, Gaylord Ellison, and Harris S. Huberman*. Dept. Psychology, UCLA, Los Angeles, CA 90024

Slow-release silicone pellets containing d-amphetamine base were implanted subcutaneously in normal rats housed in social colonies and the effects on behavior during the following seven days of constant amphetamine intoxication were observed. A reliable progression of behavioral changes was observed in 3 replications. Compared to control rats, amphetamine rats were hyperactive and exploratory on the first day after pellet implantation. This gradually evolved over 24 hours into motor stereotypies of an increasingly more circumscribed nature even though brain levels of amphetamine were equivalent to a 2mg/kg subcutaneous injection of d-amphetamine sulphate, a dose which does not produce intense stereotypy when given in acute injections. During days 2 and 3 after pellet implantation the amphetamine animals engaged in nearly constant stereotypies, self-grooming, sniffing surfaces, or manipulating straw. The stereotypies then decreased even though appreciable amphetamine was still present in the brain, and on the 4th day after implant the amphetamine animals transiently withdrew to the burrows area. Thereafter, on days 5-7, they showed heightened startle responses, increased fight or flight behavior, and stable aggressive pair-bonds formed among the amphetamine animals. This late stage of social disruptions produced by constant amphetamine intoxication can serve as an animal model of amphetamine psychosis in humans because the stages of behavior are similar to those shown by humans given frequent, low doses of amphetamine.

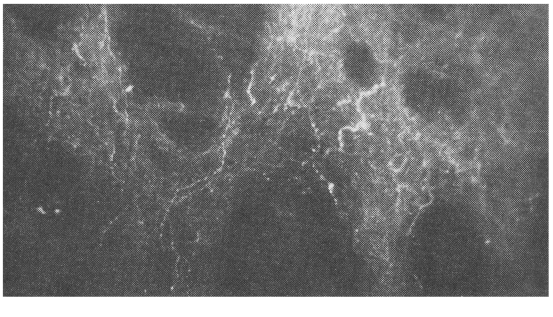
These stages of constant amphetamine intoxication are altered by radio-frequency lesions of Substantia Nigra (SN) or Locus Coeruleus (LC). Rats with SN lesions initially show enhanced locomotion but attenuated stereotypy and anorexia, while rats lesioned in LC exhibit a rapid onset of sniffing stereotypies. However, the later stages of constant amphetamine intoxication occur only in unlesioned controls, who show the most intense stereotypy on day 2, the greatest rebound depression (time in burrows), and maximally show the social disruptions which appear after 5 days of constant amphetamine. Thus, the integrity of both dopaminergic and noradrenergic systems was necessary for the full expression of this animal model of amphetamine psychosis.

778 A SLOW-RELEASE SILICONE PELLET FOR PRODUCING CONSTANT AMPHETAMINE STIMULATION: DELAYED EFFECTS ON BEHAVIOR, BIOCHEMISTRY, AND CAUDATE MORPHOLOGY. Gaylord Ellison, Harris Huberman* and Michael S. Eison. Dept. Psychology, UCLA, Los Angeles, CA 90024

The design and construction of slow-release silicone pellets containing 48mg of d-amphetamine (dA) will be described. These pellets release dA for at least 10 days, producing brain dA levels of 1.58ug/g and 0.88ug/g at 2 and 7 days after implantation in adult rats. Implanted rats become hyperactive in stabilimeters within a few hours and gradually enter continuous stereotypy for several days. After 3 days the stereotypies break and the rats become hyperirritible and startle easily. Tolerance can be shown by decreased stereotypy when fresh pellets are implanted.

Rats were perfused at various times after pellet implantation and glyoxylic acid, vibratome-sectioned slides prepared. At about the same time the stereotypies disappear there is a decrease in background fluorescence in the caudate and bright catecholamine axons with swollen varicosities appear. These effects increase with time: 17 days after implantation and 10 days after pellet removal there are long, thick fluorescing axons in the caudate and engorged, stump-like processes (see Figure below).

These alterations in caudate morphology are accompanied by distinctive regional changes in tyrosine hydroxylase activity and morphology of substantia nigra cells. These changes are similar to those reported in intact regions of the caudate after partial caudate lesions, implying that constant dA even at low doses leads to the destruction of dopamine terminals. At the same time these alterations appear the rats break out of stereotypy and begin to show paranoid-like behaviors, implying that structural alterations in the caudate are correlated with the appearance of an animal model of amphetamine psychosis.



779 MONOAMINE-CONTAINING DENDRITES: A GENERAL MAMMALIAN PHENOMENON. David L. Felten and John R. Sladek, Jr. Depts. of Anatomy, Indiana Univ. Sch. Med., Indianapolis, IND. 46202 and Univ. Rochester Sch. Med. and Dent., Rochester, N.Y. 14642.

Primate and non-primate monoamine systems were examined for the presence of monoamine-containing dendrites with the Falck-Hillarp and the glyoxylic acid methods. Monoamine-containing dendrites were found in all major monoamine groups in unpretreated rhesus, stump-tail, and squirrel monkeys, and were also abundant in monoamine groups in the rat, cat, and rabbit. These dendrites were both smooth and varicose in appearance. Some fluorescent dendrites formed bundles with close apposition of individual processes.

Norepinephrine-containing dendrites. Neurons of the medullary and pontine noradrenergic groups were multipolar with numerous fluorescent primary and secondary dendrites. Groups A1 and A5 demonstrated the most extensive dendritic profiles. Groups A6, A7 and central gray noradrenergic cells also displayed prominent dendritic fluorescence. Locus coeruleus dendrites bundled together transversely as they traversed the mesen. tract. V.

Dopamine-containing dendrites. Neurons of groups A8, A9 and A10 displayed prominent dendritic fluorescence despite major architectural variations in the appearance of these groups in the background of fluorescent processes superimposed on the cell bodies. Dendrites were frequently seen extending ventrally into pars reticulata of substantia nigra. A10 dendrites occasionally bundled among emerging fibers of the III nerve. Dopamine-containing dendrites were also present in the arcuate nucleus and other hypothalamic cell groups, but were best seen in neonates with the glyoxylic acid method.

Serotonin-containing dendrites. Serotonin dendrites of both smooth and varicose appearance were visible in all serotonergic groups, but were strikingly abundant in the dorsal raphe nucleus and central superior nucleus and in laterally adjacent reticular formation. Serotonergic dendrites formed prominent bundles within the medial longitudinal fasciculus.

This study confirms the finding of fluorescent dendrites reported in substantia nigra by Björklund and Lindval (BRAIN RES. 83:531, 1975) and in locus coeruleus by Sladek and Parnavelas (BRAIN RES. 110:657, 1975). It expands Felten's findings of the pervasive presence of monoamine dendrites in the squirrel monkey (BRAIN RES. 120:553, 1977) to other primates and non-primates. The presence of monoamines within dendrites may reflect nothing more than a storage compartment for parikaryal synthesis of these transmitters. Another possibility based upon recent evidence for release and post-synaptic activity of monoamines from dendritic areas is that dendritic neurotransmission, or simply dendritic release may be a generalized mammalian phenomenon.

780 SENSITIVITY CHANGES IN THE CENTRAL CARDIOVASCULAR ACTIONS OF SEROTONIN. E. Friedman*, E. Buchweitz* and G. Lambert* (SPON: D. Quartermain). New York Univ. School of Medicine, New York, NY 10016.

In previous studies from our laboratory, we have characterized the centrally-mediated cardiovascular responses to serotonin-receptor stimulation in the rat (Life Sci. 17, 915, 1975). These appear to be localized in an area perfused by the third ventricle. In the present communication, we report on experiments designed to explore possible alterations in receptor-sensitivity in this serotonin neuronal system. In the first set of experiments, serotonin-containing neurons were destroyed by intraventricular (ivc) injection of 75 ug of 5, 6-dihydroxytryptamine (DHT) and receptor sensitivity was monitored 3-4 weeks later. The extent of neuronal destruction was followed by measurements of ^{3}H-serotonin uptake into hypothalamic synaptosomes obtained from DHT-treated and control-vehicle-treated rats. The dose-response curve to ivc injection of serotonin in the lesioned rats showed a 1000 fold shift to the right when compared to sham animals. However, the responses to ivc injection of 2, 5 dimethoxy 4-methyl amphetamine (DOM), a direct serotonin receptor agonist, into lesioned animals did not differ from those in sham rats. In a second series of experiments, receptor sensitivity changes were monitored following repeated serotonin-receptor stimulations. Decrease in receptor sensitivity was noted during repeated injections of serotonin or DOM. The degree of sensitivity loss with serotonin developed slowly at low doses (50 ng-5 ug) and more rapidly at higher doses. The desensitization to ivc DOM was more noted. These results suggest that (1) the enhanced sensitivity to ivc serotonin observed following destruction of central serotonergic neurons is due to interference with the serotonin uptake process and not to alterations at the receptor level and (2) receptor desensitization develops to repeated serotonin receptor stimulation.

Supported by a Grant-in-Aid from the American Heart Association to E.F.

781 ELECTROPHYSIOLOGICAL RESPONSES OF MIDBRAIN DOPAMINERGIC NEURONS TO A NON-AMPHETAMINE CNS STIMULANT. D. C. German, H. Harden*, S. Browder*, F. Morrison*, R. S. Kiser, and P. A. Shore*. Depts. of Physiol., Psychiat., and Pharmacol., U. of Texas Health Sci. Ctr., Dallas, TX, 75235.

Amfonelic acid (AFA) is a potent CNS stimulant. Unlike d-amphetamine (d-AMP), AFA has little peripheral action, and AFA's central action in the rat is not blocked by inhibition of tyrosine hydroxylase, but is attenuated by reserpine. d-AMP is thought to act on the dopamine (DA) neuron by direct release of DA and blockade of reuptake, while AFA acts on the DA neuron to facilitate impulse-induced DA overflow (Shore, J. Pharm. Pharmac., 28(1976), 855-857). Because d-AMP is known to reduce the firing rate of midbrain DA neurons, an effect reversed by a subsequent injection of haloperidol (Hal) (Bunney et.al., J. Pharmacol. Exp. Ther., 185(1973), 560-571), it was of interest to determine whether AFA had similar effects on DA cell firing rates. Recordings were made from over 100 rats; one cell per rat. In the chloral hydrate anesthetized rat, single units recorded in the DA regions of the ventral tegmental area and substantia nigra zona compacta (histologically confirmed) had baseline firing rates of 1-7 Hz. Single units recorded outside these areas (i.e., in reticular formation, red nucleus, zona reticulata) had more variable firing rates, from 2-32 Hz. The firing rates of the cells in the DA regions were decreased by greater than 50% of their baseline firing rates within 5-15 min after an injection of either d-AMP (1 mg/kg) or AFA (1 mg/kg). This reduction by either drug was reversed by a subsequent injection of Hal (1 mg/kg), and the cells returned to or above baseline firing rates within 10 min. The firing rates of the cells outside the DA regions did not respond to the drugs as did those in the DA regions. Thus, although AFA has a different mechanism of action on the DA neuron, it mimics d-AMP in decreasing DA cell firing rates and having this decrease reversed by the DA receptor blocker, Hal.

(Research supported by NIMH Grants MH-27574 and MH-05831).

782 DEMONSTRATION OF BULBOSPINAL NORADRENERGIC NEURONS BY LOCALIZATION IN THE SAME CELL OF BOTH DOPAMINE-β-HYDROXYLASE (DBH) AND RETROGRADELY TRANSPORTED HRP. E.J.Glazer, A.J.Smolen, L.L.Ross, T.H. Joh, V.M.Pickel and D.J. Reis. Dept. Anat., Med. Coll.of Penna.,Phila.,Penna.,19129, Lab.Neurobiol.,Dept.Neurol., Cornell Med. Coll., N.Y.., N.Y. 10021.

In a previous study we have traced the descending noradrenergic input to the sympathetic lateral columns in rat thoracic cord by the immunocytochemical localization of DBH. Noradrenergic axons were observed in the dorsolateral funiculus which terminated predominantly within both ipsilateral and contralateral intermediolateral cell nuclei.

The purpose of this study was to identify the brainstem noradrenergic neurons which project to the spinal cord. This was accomplished by the localization of both DBH and retrogradely transported horseradish peroxidase (HRP) within the same cell. The technique employed was as follows: Sixteen to twenty-four hours following a midthoracic injection of HRP (4 ul 33% HRP, Sigma Type VI) animals were fixed by perfusion and the brainstems serially sectioned at 30 μ on a Vibratome. The sections were then reacted for HRP using benzidine dihydrochloride, H_2O_2 and sodium nitroferricyanide as substrates which yield a blue reaction product. (Mesulam, M., J. Histochem. Cytochem. 24:1273, 1976). Every third section was mounted in glycerin and labeled neurons were photographed. The coverslip was removed and DBH was localized on the same section by the unlabeled antibody enzyme method and PAP complex. The diaminobenzidine-H_2O_2 reaction, which produces a brown precipitate, was used to localize the PAP In addition, adjacent sections were reacted for either transported HRP or DBH.

Neurons with both labels were identified in both the A1 and A2 noradrenergic cell groups in the caudal medulla extending back to the level of the motor decussation. The A1 cell group consisted of a loose network of cells in the ventrolateral reticular formation. Neurons within the A2 cell group were observed in the lateral margins of the commissuralis portion of nucleus solitarius. It should be noted that not all HRP labeled cells in the ventral reticular formation contained DBH. Similarly HRP labeled non-adrenergic neurons were observed in the A2 area and probably represent a solitario-spinal projection. Our method of labeling DBH and retrogradely transported HRP in the same cell enables us to map out bulbo-spinal neurons which synthesize norepinephrine.

Supported by NIH (NSO5195, NSO5392, NS11364 and HL18974).

783 PHARMACOLOGICAL ACTIVATION AND INHIBITION OF NORADRENERGIC ACTIVITY ALTER SPECIFIC BEHAVIORS IN NONHUMAN PRIMATES. Mark S.Gold, and D.E. Redmond, Jr., Dept. Psychiatry, Yale Univ., New Haven,Ct.

Four rigorously chair-trained and acclimatized *M. arctoides* were recorded on split-image videotape with superimposed computer-generated timing and event coding for later blind behavioral rating of sequential and simultaneous behaviors from an ethogram for each one second period. 1 and 2.5 mg/kg of piperoxane but not saline administered through an indwelling venous catheter from outside a sound dampened cubicle increased specific behaviors with a time course that closely approximates the onset of increases in single unit activity in the rat locus coeruleus (LC) after identical doses/body weight (Cederbaum and Aghajanian, BR. RES. 112: 413, 1976). 10 µg/kg clonidine reduced baseline behaviors, prevented many of the behavioral effects, and transiently reversed the behavioral effects of 1 mg/kg piperoxane. Clonidine's onset of behavioral effects also corresponds to the decreased unit activity seen in the rat LC after a similar dose of clonidine (Svensson et al., BR. RES. 92:291, 1975). Epinephrine (E)-containing neurons have been shown to innervate the LC (Hokfelt et al, BR. RES. 66: 235, 1974). In the doses used in this study it is believed that clonidine stimulates and piperoxane inhibits, inhibitory E or norepineprine-responsive "auto" receptors on the cell bodies of the LC to decrease and increase noradrenergic firing rate, release and turnover respectively (Bolme et al, EUR. J. PHARM., 28:98, 1974; Maas et al, BR. RES. 118:167, 1976). Although these drugs also have effects on other neurotransmitter systems, the behavioral effects are possibly relevant to changes in LC activity, as behaviors which are increased by piperoxane are increased by low intensity electrical stimulation of the LC (Redmond, et al, BR. RES. 116: 502, 1976),and behaviors reduced by clonidine are decreased by bilateral lesions of the LC (Huang et al, NEUROSCI. ABS., 1976; Redmond et al, NEUROSCI. ABS. 472, 1976). Behaviors induced by piperoxane administration in monkeys may be relevant to human anxiety since piperoxane has been reported to induce anxiety in humans (Goldenberg et al, JAMA 135: 971, 1947; Soffer, MED. CLIN. N. AM. 38: 375, 1954). Details of the similarities between the behavioral effects of electrical stimulation of the LC and intravenous piperoxane will be presented by Huang, et al.

784 THE EFFECTS OF ESTROGEN ON DOPAMINE TURNOVER, GLUTAMIC ACID DECARBOXYLASE ACTIVITY AND LORDOSIS BEHAVIOR IN SEPTAL LESIONED FEMALE RATS. J. H. Gordon, D. M. Nance, C. J. Wallis* and R. A. Gorski. Dept. Anat. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

As reported in previous studies, estradiol benzoate (EB) treatment (2.0 µg/day X 3) of septal lesioned (SL) adult female rats produced a marked increase in lordosis quotient (LQ; 66±3) over that of similarly EB primed sham operated (SO; 19±3) animals. Endogenous dopamine (DA) and norepinephrine (NE) were measured and turnover rates were estimated by the rate of decline in endogenous amine following tyrosine hydroxylase inhibition with α-methyltyrosine. No consistent pattern in the level or rate of turnover of NE was noted for any of the treatment groups. No differences in either the level or turnover of DA were seen in the oil treated SL group, relative to SO groups. In the amygdala, corpus striatum, and nucleus accumbens septi of EB treated SL animals there was both a reduction in endogenous levels (20-50%) and a decrease in the synthesis rate (Table 1) of DA relative to both SO groups and oil treated SL animals. In the EB treated SL animals there was a significant negative correlation between DA levels in the corpus striatum and LQ (r = -.850), which is consistent with the proposed inhibitory action of DA on sexual receptivity. Because of the apparently generalized reduction in DA turnover in the EB treated SL animals, glutamic acid decarboxylase (GAD) activity in the substantia nigra (SN) and ventral tegmental region (VTR) was measured to test for any possible alterations in the proposed inhibitory neuronal feedback. GAD activity was reduced in both the SN and VTR of EB treated SO animals (Table 1) but remained comparable to oil treated SO controls in both EB and oil treated SL animals. These data suggest that the reduction in DA turnover, in the EB treated SL animal, may be due to a lack of a compensatory decrease in GAD activity in the regions containing the DA cell bodies. (Supported by Grant HD-01182 and the Ford Foundation.)

TABLE 1: NEUROCHEMICAL ALTERATIONS IN SEPTAL LESIONED RATS

	Sham Operated		Septal Lesioned	
Treatment	Oil	EB	Oil	EB
DA Synthesis Rate				
Corpus striatum	23.19*	20.38	25.86	13.11+
N accumbens septi	17.17	16.55	16.33	11.10
Amygdala	0.64	0.81	0.55	0.22+
GAD Activity				
Substantia nigra	375±24**	196±5+	437±10	356±21+
Ventral tegmentum	143±10	101±5+	157±12	162±6

*Values expressed as pmole/mg/hr; **Values are mean ± S.E. in nmoles/mg/hr; +Significantly reduced from oil treated group.

785 AGE DEPENDENT ALTERATIONS IN DOPAMINERGIC MECHANISMS WHICH ACCOUNT FOR d-AMPHETAMINE'S PARADOXICAL EFFECTS UPON SEIZURE SUSCEPTIBILITY IN SELECTED LINES OF MICE. Charles A. Greer and Herbert P. Alpern. Dept. Psych. and Inst. Behav. Genet., Univ. Colo., Boulder, CO 80309.

A developmental analysis of the effect of d-amphetamine (AMPH) on flurothyl-induced myoclonic convulsions was conducted in two lines of mice (Long-Sleeps [LS] and Short-Sleeps [SS]) which had originally been selectively bred on the basis of susceptibility to ethanol induced narcosis. A total of eight different ages were tested ranging from 15 to 250 days of age. The LS line exhibited minimal developmental changes with AMPH consistently producing a proconvulsant effect. In marked contrast, the SS mice responded with a decrease in seizure susceptibility following AMPH until approximately 80 days of age. The subsequent proconvulsant effect of AMPH in the SS line was essentially equal in magnitude to that seen in the LS line by 120 days of age and remained stable through 250 days of age. In an attempt to determine whether dopamine (DA) or norepinephrine (NE) was mediating this marked alteration in response, either apomorphine, haloperidol, clonidine, or phentolamine plus sotalol was administered to mice 30 and 120 days of age in both lines. Clonidine and apomorphine produced a proconvulsant effect in both age groups of LS mice with a complementary anticonvulsant effect following the respective DA and NE antagonists. In the young SS mice however, while NE compounds produced effects comparable to that seen in the LS line, apomorphine produced an anticonvulsant effect reminiscent of that seen with AMPH while haloperidol resulted in the complementary increase in seizure susceptibility. The 120 days old SS mice, however, exhibited a marked increase in seizure susceptibility following apomorphine with the concomitant decrease following haloperidol while NE manipulations did not significantly alter myoclonic latencies.

These findings clearly reveal a significant developmental alteration in dopaminergic mechanisms in the SS line. Currently, biochemical studies are being initiated in order to elucidate the precise nature of these alterations. Finally, it is of value to note the similarity of the phenomenon described here to the hyperkinetic syndrome in children and its clinical management, in some cases, with AMPH.

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786 ANALYTICAL MICROSCOPY OF AMINE POSITIVE STRUCTURES IN PRIMATE SUBSTANTIA NIGRA NEURONS. David Harling , and Joe Wood. JEOL, USA, Medford, MA; Dept. of Neurobiology and Anatomy, Univ. of Tex. Med. Sch. at Houston, Houston, Tex. 77025.

The dopamine (DA) positive neurons of the substantia nigra (SN) have been identified in a number of animals by histofluorescence techniques; however, electron microscopic localization of the DA organelles in SN neurons has been elusive. With use of the glutaraldehyde-dichromate (GDC) technique, biogenic amines have been identified in a number of areas within the central and peripheral nervous systems. Monkeys were perfused with 3% glutaraldehyde (pH 7.2). The SN areas were dissected and treated with GDC solution (pH 4.1). Some glutaraldehyde fixed material was incubated in 2% silver chloride, modified after Tramezzani et al (1964) and other glutaraldehyde fixed tissues were embedded without metal treatment. Electron microscopy shows large (4000 Å) irregularly shaped densities in the cell bodies and processes of SN neurons. These densities were observed in perikarya, axons and dendrites. Analytical electron microscopy of these same structures according to Harling and Wood (1975) produced high chromium (Cr) peaks indicative of the presence of DA. These Cr peaks were compared against chromium crystals and a model system of glutaraldehyde plus DA and potassium dichromate. Model spectra were similar when compared against readings from Cr positive structures with the exception that there was a high sulfur (S) content in the neuronal structures. Many SN dense bodies are suspect of being pigment bodies, therefore, sections were stained with uranyl acetate and/or lead citrate. The amine-Cr positive structures appear different from other nonpositive densities which resemble pigment. Similar results were obtained with silver chloride with the exception that the silver chloride was less consistent and produced reactive artifact. The Cr positive deposits are similar in some respects to those seen previously in the arcuate nucleus but in other respects there are differences in ultrastructural configurations. These results show positive ultrastructural indentification of DA in the dopaminergic neurons of the SN. Also these Cr positive structures are not limited to certain cell areas since they are in perikarya, in dendrites and possibly in axons. Reserpine diminishes the number and size of densities as well as the Cr content. The other possibility exists, that pigment granules exist in different states and that pigment granules may contain DA. In vitro studies using melanin instead of DA produce no reaction. Now that a positive DA method is available, the ultrastructure of the DA storage site can be determined and with analytical electron microscopy, the elemental characteristics of these sites can be evaluated. (Supported by the Salk Foundation of Texas and by Grant #NS10326.)

787 MONOAMINE OXIDASE A AND B IN CULTURED CELLS. Morris Hawkins, Jr* and Xandra O. Breakefield* (SPON: E.L. Giller). Dept. Human Genetics, Yale Univ. Sch. of Med., New Haven, CT 06510.

The degradative deamination of tryptamine by monoamine oxidase (MAO) was compared in several continuous cell lines of rodent origin, including neuroblastoma, hepatoma, melanoma, nephroma, sarcoma and L cells. Homogenates of stationary phase cells show a 300-fold variation in activity against [2-^{14}C]tryptamine with the highest specific activity being 1043 pmol/min/mg/protein in hepatoma line MH_1C_1 and the lowest being 3 pmol/min/mg/protein in neuroblastoma line N1E-115TG2 which lacks hypoxanthine phosphoribosyltransferase (HPRT). Levels of MAO activity vary with the stage of growth in culture. MAO activity was lower in 3 lines lacking HPRT activity as compared to the parental lines from which they were derived; activities were 100-fold lower in N1E-115TG2, and 3-fold lower in both N-18TG2 and PC1A-HPRT$^-$, however, no difference was found between MAO activities in HTC and HTCPRT$^-$ cells. Succinate-cytochrome C reductase (SCCR), another mitochondrial enzyme, showed a 20-fold variation in activity between cell lines, being highest in neuroblastoma line N1E-115 and lowest in hepatome line MH_1C_1, with values of 49 nmol and 2 nmol/min/mg protein, respectively. SCCR and MAO activities appeared to be regulated independently. Clorgyline (1 nm), a selective inhibitor of A type MAO activity, completely blocked deamination of tryptamine in homogenates of all lines except MH_1C_1. Using either clorgyline or deprenyl, a selective B inhibitor, MAO activity in MH_1C_1 homogenates appeared to be 30-60% of the B type and 40-70% of the A type. In neuroblastoma N1E-115 cells in culture, clorgyline (10 nm) completely inhibited MAO activity against tryptamine and dopamine, indicating that the A type of MAO is active intracellularly as in homogenates. In hepatoma MH_1C_1 cells in culture tryptamine deamination, in contract to that seen in homogenates, showed exclusive B type activity.

We conclude that: 1) homogenous populations of a number of peripheral, rodent cell types possess almost exclusively the A type of MAO activity against tryptamine, as measured in homogenates by sensitivity to clorgyline; 2) the lowering of MAO activity seen with HPRT deficiency may depend on factors of intermediary metabolism which vary between lines, or may reflect different mutational or epigenetic events leading to HPRT deficiency; 3) total MAO activity is regulated in culture with the stage of growth and varies independently of at least one other mitochondrial enzyme, SCCR; 4) in mouse neuroblastoma clone N1E-115, MAO activity of the A type against tryptamine and dopamine occurs in living cells, as in homogenates; and 5) in rat hepatoma line MH_1C_1 cells under conditions of growth tryptamine metabolism is mediated by B type activity, but homogenization leads to expression of A type of activity.

788 EVIDENCE FOR NORADRENERGIC SPECIFICITY OF BEHAVIORAL EFFECTS OF ELECTRICAL STIMULATION OF THE NUCLEUS LOCUS COERULEUS. Y.H. Huang, J.W. Maas, and D.E. Redmond, Jr., Dept. Psychiatry, Yale Univ., New Haven, Ct. 06510.

The behavioral effects of low level electrical stimulation of the locus coeruleus (LC) in chair-trained *M. arctoides* were studied after agents which activate or block the functional effects of the locus coeruleus. 10 µg/kg clonidine, which is thought to block the E or NE receptors on the cell bodies of the locus coeruleus, blocks the behavioral effects of electrical stimulation of the locus coeruleus with bipolar, biphasic stimulation (from 0.4 to 0.5 mA intensity, 0.5 msec square wave pulses, and 10-30 Hz frequency). 5 mg/kg of propranolol, a post-synaptic ß-adrenergic antagonist also abolishes the effects of stimulation of the locus coeruleus. Electrical stimulation of the locus coeruleus at these parameters cannot be regularly distinguished from the effects of threatening or frightening situations. All produce mouth movement, scratching, yawning, hand wringing, body jerks, and other behaviors in a pattern which is consistent within animals between the various eliciting stimuli, but differs somewhat between animals, *i.e.* monkeys which hand wring in response to threats also hand wring after piperoxane administration or electrical stimulation of the locus coeruleus. The pharmacologic specificity of the agents which block electrical stimulation of the LC, and the apparent equivalence of the behavioral effects of piperoxane and electrical stimulation of the locus coeruleus support locus coeruleus-noradrenergic mediation of the behavioral effects described here and in the previous paper by Gold and Redmond.

789 DESCENDING MONOAMINERGIC PATHWAYS IN THE ADULT OPOSSUM (DIDELPHIS VIRGINIANA). A.O. Humbertson, Jr., K.A. Crutcher and G.F. Martin.

Catecholamine (CA) and indolamine (IA) varicosities are found at all levels of the spinal cord in the adult opossum. Indolamine terminals are present throughout the gray matter, but are concentrated within the medial ventral horn at cervical levels and the lateral horn at thoracic and upper lumbar levels. Catecholamine terminals are extensive within the dorsal part of the dorsal horn at all levels as well as within the lateral horn. The region surrounding the central canal, throughout its length, is rich in terminals of both types.

Following horseradish peroxidase (HRP) injections of either lumbar, thoracic or cervical cord levels, labelled neurons are present within the nucleus coeruleus, the nucleus coeruleus: pars alpha (Oswaldo-Cruz, Rocha, Miranda, '68), the solitary nucleus and the lateral reticular formation. These areas contain fluorescent cell bodies of the (CA) type in the opossum. Reactive neurons also appear within the pontine and medullary raphe nuclei where fluorescent neurons of the (IA) type are present. In addition, following thoracic (HRP) injections there are labelled neurons in the periventricular nucleus as well as in dorsal and caudal regions of the hypothalamus. Although fluorescent neurons of the (CA) type are present in those areas, they are sparse.

In several cases ^{3}H-amino acids were deposited within those brainstem regions which label in the HRP experiments and which also fluoresce for monoamines. In such cases axons are labelled in regions of the spinal cord which contain fluorescent varicosities when processed by the Falck-Hillarp method. (Supported by USPHS Grants NS-10165 and NS-07410.)

790 CHANGES IN MONOAMINE OXIDASE ACTIVITY IN RABBIT PLATELETS AND BRAIN REGIONS DURING PREGNANCY. S. Huprikar*, A. Mosnaim, L. Black Jones*, G. Oltmans, V. Nair, and E. A. Zeller. Department of Pharmacology, School of Graduate and Postdoctoral Studies, University of Health Sciences/The Chicago Medical School, Chicago, IL 60612, and Department of Biochemistry, Northwestern University Medical School, Chicago, IL 60611.

Evidence for the regulation of monoamine oxidase (MAO; E:C:1:4:3:4) activity in brain and other tissues by steroid hormones has been presented by a number of investigators (Zeller et al., J. Neural Transm., in press). Since the status of these hormones is dramatically changed in pregnancy, it was of interest to study the influence of this condition on platelet and brain MAO activity. New Zealand white rabbits (3 kg; ∿10 months old) were used in these studies. Using ^{14}C-labeled phenylethylamine and serotonin as substrates for MAO types B and A respectively, we now report that during the course of pregnancy the activity of platelet MAO_B steadily decreases, reaching lowest values at term (30-32 days; controls 2.41±0.24, pregnancy 1.49±0.14 nmoles/mg protein/hr), whereas MAO_A showed slight but significant increase (control 1.10±0.05, pregnancy 1.37±0.04 nmoles/mg protein/hr). At term, the activities of both MAO_B and MAO_A did not change in either the caudate, hippocampus, hypothalamus, or cortex, while they were significantly increased in the brainstem (control MAO_B 1995±605, pregnancy MAO_B 3684±212 nmoles/g wet tissue/hr, $p<0.05$; control MAO_A 1147±345, pregnancy MAO_A 3945±1050 nmoles/g wet tissue/hr, $p<0.01$). An increase for MAO_A was also observed in cerebellum (control MAO_A 1402±345, pregnancy MAO_A 2466±383 nmoles/g wet tissue/hr, $p<0.01$). These results suggest the involvement of the gestational hormones-MAO system in the regulation of biogenic amines during pregnancy. (Supported in part by Shriners Hospital for Crippled Children, Chicago, and by Biomedical Research Support Grant RR-05366, NIH.)

791 DIFFERENTIAL PROJECTIONS OF NEURONS WITHIN THE DORSAL RAPHE NUCLEUS OF THE RAT: A HORSERADISH PEROXIDASE (HRP) STUDY. Barry L. Jacobs, Stephen L. Foote and Floyd E. Bloom. Princeton Univ., Princeton, NJ and Salk Inst., La Jolla, CA.

The efferent projections of the midbrain raphe nuclei dorsalis (DR) and medianus (MR) in the rat have previously been reported to be differential (Jacobs, et al. Brain Res. 1974, Vol. 79, p. 353). The present study examined whether subareas within the DR have different projections, i.e. whether the nucleus is topographically organized. Since the raphe nuclei are symmetrically shaped midline structures, we also examined whether the projections of their constituent neurons were unilateral or bilateral. Unilateral injections of 30% HRP (0.1 - 0.5ul) were placed in one of three areas: dorsal neocortex-hippocampus, corpus striatum, or amygdala-piriform cortex of adult male albino rats. The animals were perfused 24 hrs later with a mixture of paraformaldehyde and glutaraldehyde and the brains were then removed and stored in sucrose buffer for 24 hrs. The brains were then cut (40u thick sections), reacted with 3-3' diaminobenzidine hydrochloride, mounted, cover slipped, and examined with both light and dark field microscopy. The diffusion of HRP at the injection site was often quite extensive, however, in the animals selected for analysis, there was never any overlap of the three injection sites. Some portions of the DR, e.g. the ventromedial cluster of cells, contained HRP positive cells which were common to the three injection sites. On the other hand, some labelled sites within the DR were unique to a particular injection site. For example, only injections into the amygdala-piriform cortex labelled cells in the most posterior aspect of the DR and its caudal extension into B-6. Amygdala injections also yielded more heavily labelled cells in the dorsomedial cluster of the DR than did injections in either of the other two sites. Projections of DR cells are quite lateralized, as evidenced by the appearance of the most heavily labelled cells ipsilateral to the injection site. Some contralateral labelling, especially of cells close to the midline, was also evident. By comparison, cells in the MR appeared to be less lateralized. These data also confirmed our previous studies on the differential projections of DR and MR since we found that MR cells were heavily stained for HRP after dorsal cortex-hippocampal injections and contained little or no HRP positive cells following striatal injections which did consistently label cells in the DR. In conclusion, DR neurons have largely ipsilateral projection fields which are somewhat different from the projection sites of cells located in other areas of the DR as well as from cells in the MR. A previously unreported projection to the amygdala-piriform cortex from B-6 was also observed. (Supported by NIMH grant MH-23433 and NSF grant BNS 76-09318).

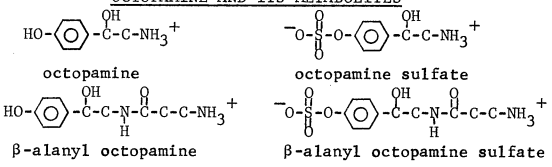
792 AMINE METABOLISM: A DIFFERENT PATHWAY IN LOBSTERS. Mary B. Kennedy* (SPON: E. A. Kravitz). Harvard Med. Sch., Boston, MA 02115.

Previous work from this lab has shown that lobster tissues can take up and metabolize octopamine, dopamine, and serotonin, the three major amines found in lobsters. Yet the tissues do not contain catechol-O-methyl transferase or monoamine oxidase, the enzymes that metabolize the amines in mammals. This report is concerned with the pathway of amine metabolism in lobsters.

Sulfate conjugates: When lobster leg nerves were incubated with ^{3}H-amines, three ^{3}H-products were formed from each amine. Two of the three products were labelled with ^{35}S when $^{35}SO_4$ was also included in the incubation medium. Hydrolysis of one double-labelled octopamine product produced a mixture of $^{35}SO_4$, and ^{3}H-octopamine. Hydrolysis of the other produced $^{35}SO_4$, and varying amounts of ^{3}H-octopamine and a ^{3}H-compound with the electrophoretic mobility of the third, non-sulfate containing, product. This suggested that sulfate conjugates were formed both from octopamine and from the third metabolite. Properties of the products indicated that the SO_4 was esterified to the ring hydroxyl groups. An enzyme system that catalyzed the formation of amine sulfates from the three amines and phosphoadenosine phosphosulfate was found in homogenates of the nerves. Thus, one major pathway of amine metabolism in lobsters is sulfate conjugation.

β-Alanine conjugates: The octopamine conjugate that did not contain SO_4 could be converted back to octopamine by hydrolysis, indicating that it was also a conjugate. The metabolite was reacted with dansyl chloride (DNS-Cl). Hydrolysis of the resulting DNS-derivative produced a DNS-compound that co-chromatographed with DNS-β-alanine in four solvent systems. This suggested that the metabolite was a β-alanine conjugate. Its characteristics indicated that the β-alanine was linked by an amide bond to the amino group of octopamine. An enzyme system that catalyzed the formation of amine products from each of the amines, ATP, and β-alanine was present in leg nerve homogenates. These results suggest that a second major pathway of octopamine metabolism, and possibly of amine metabolism in general in the lobster, is β-alanine conjugation. Supported by NIH.

OCTOPAMINE AND ITS METABOLITES



793 EFFECTS OF REPEATED IMMOBILIZATION STRESS ON PLASMA LEVELS OF EPINEPHRINE, NOREPINEPHRINE AND DOPAMINE-β-HYDROXYLASE. R. Kvetňanský*#, C.L. Sun*, T. Torda*#, C.R. Lake and I.J. Kopin, (SPON: O.H. Viveros).
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Repeated immobilization (IMO), daily for 2.5 hrs., increases adrenal medullary catecholamines (CA) and CA-synthesizing enzymes and enhances urinary excretion of CA over the increase produced by a first IMO. Decapitation results in a sudden massive activation of the sympatho-adrenal medullary system with striking increases in plasma levels of CA. Rats decapitated after repeated IMO had greater increases of plasma CA during the first 5 minutes of IMO than did rats IMO for the first time. Repeated IMO for 150 min. daily had a greater effect on increasing levels of plasma CA, in rats decapitated 24 hours after the last IMO, than did IMO for 10 min. daily. The enhancement of the increase in CA produced by decapitation was greatest after 7 days of repeated IMO, but was still significantly enhanced after 45 days. Adrenalectomy resulted in virtual absence of epinephrine in plasma both before and during IMO, while plasma norepinephrine was not essentialy changed. In blood obtained from an indwelling arterial cannula, levels of epinephrine and norepinephrine were much lower than after decapitation (0.07 ng vs. 7.00 ng E and 0.25 ng vs 2.20 ng NE/ml of plasma). IMO resulted in striking increases in plasma levels of both CA, but there were greater increases during the first IMO than during IMO after the 30th repeated exposure. In undisturbed rats, however, basal levels (24 hrs. after the last IMO) of epinephrine and norepinephrine were greater in animals subjected to repeated IMO. Plasma levels of dopamine-β-hydroxylase were higher in repeatedly IMO rats (24 hours after the last IMO) than in unstressed animals. Furthermore, during IMO, levels of the enzyme were increased to a greater extent in the repeatedly stressed rats.

Thus, basal levels of epinephrine and norepinephrine and dopamine-β-hydroxylase activity in plasma are increased when the capacity to synthesize CA is enhanced by repeated IMO stress. This is also reflected by the maximal CA release which follows decapitation. After rats are subjected to repeated IMO, however, although the capacity to discharge CA is increased, plasma levels of CA are not increased to the same extent as in IMO naive rats, presumably as a consequence of reduced CNS response to IMO in the experienced animals.

794 NO DIFFERENCE IN CEREBROSPINAL FLUID LEVELS OF NOREPINEPHRINE IN PATIENTS WHEN ON HIGH VERSUS LOW MONOAMINE DIETS. C.R. Lake, M.G. Ziegler*#, I. Shoulson## and I.J. Kopin. NIMH, Bethesda, MD 20014.
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In research protocols in which catecholamines are to be determined, the subjects are often maintained on a low monoamine (LMA) diet. Urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of norepinephrine (NE) reflecting brain NE metabolism, was found to be significantly decreased in depressed patients but unchanged in normal volunteers by the LMA diet, but effects of diet on CSF levels of amines have not been examined. We measured levels of NE in cerebrospinal fluid (CSF) from 5 aliquots of 4 ml each and from plasma and noted blood pressure and pulse rate from each of 6 patients with Huntington's disease while on a LMA diet and again while on a high monoamine (HMA) diet. Huntington's patients had significantly lower levels of NE when compared to age matched controls. Levels of NE in CSF first removed from the lumbar tap needle may have contributions from spinal nerve terminals while NE from higher aliquots of CSF are thought to reflect brain metabolism. When NE from each CSF aliquot was examined using the paired Student t-test, levels of NE were higher while subjects were on the LMA diet but only the second aliquot (ml 4 through 8) was significantly ($p < 0.01$) higher. When CSF levels from all aliquots were compared on the two diets by analysis of variance, there were no differences between CSF levels of NE on the 2 diets. The means ($\pm$ SEM) of all aliquots of all patients were 141 $\pm$ 11 pg/ml and 150 $\pm$ 10 while on the HMA and LMA diets, respectively. Supine plasma levels of NE on the HMA diet was 243 $\pm$ 65 pg/ml and on the LMA diet was 320 $\pm$ 19. Blood pressure and pulse rate were similar on the two diets. The lack of difference in CSF levels of NE under the two dietary conditions presumably reflects an efficient hepatic removal and effective blood-brain barrier for NE. Thus, dietary, restrictions may be unnecessary when examining CSF for NE.

795 THE RELATIONSHIP BETWEEN PLASMA CATECHOLAMINES, CORTISOL, PSYCHOLOGICAL FACTORS AND DURATION OF PARTURITION. Regina Lederman*, Daisy S. McCann, Wayne County General Hospital, Eloise, Mi 48132 & the Depts Nursing and Internal Medicine, The University of Michigan, Ann Arbor, Mi 48109

In last trimester pregnancy measures of plasma catecholamines and cortisol, self-report anxiety, and psychological factors relevant to parturition were obtained from 32 primigravidas, 20-32 years old, with no obstetrical complications. During labor, self-report anxiety and the biochemical measures were also obtained at the onset of first stage active labor and second stage labor. These data were analyzed for relationships to uterine activity (Montevideo Units) and length in each phase of labor.

Poor relationships with the subject's husband and mother, high fears pertaining to pain, helplessness, the loss of self-control and self-esteem, low acceptance of pregnancy, and a history of psychological counseling were significantly correlated with lower uterine activity during active labor. Fears of pain and helplessness, poor identification of a motherhood role, and low acceptance of pregnancy were significantly related to longer active phase labor. The latter two variables were also significantly related to longer second stage labor. Self-report anxiety in pregnancy correlated with the two anxiety measures in labor and with uterine activity in active labor.

During labor the biochemical measures were significantly elevated, but statistically unrelated to one another. The anxiety measure in active labor correlated with epinephrine (E), $r = .60$ ($p<.01$) and with cortisol, $r = .59$($p<.01$), but not with norepinephrine (NE). E and cortisol also correlated significantly and positively with uterine activity, but only E correlated with the length of active labor, $r = .60$($p<.01$). In second stage labor all the biochemical measures correlated positively and significantly with the anxiety measure, but only E correlated with the length of second stage labor, $r = -.30$($p<.05$). There are also significant correlations between first stage active and second stage labor for NE, uterine activity and length of labor, but not self-report anxiety, indicating that progress in the two phases of labor are related, but the determinants of anxiety are different in each phase.

The data reported are consistent with the hypothesis that there are relationships among anxiety, selected biochemical measures, and progress in labor, and suggest a need for a program of early diagnosis and intervention of anxiety.

796 CELLULAR PROPERTIES OF THE MONOAMINE NEURONS IN THE LEECH CNS. Charles M. Lent and Bryan M. Frazer*. Dept. Biology, SUNY Stony Brook, New York 11794.

Nine widely-scattered neurons in leech segmental ganglia contain biogenic monoamines (MA). The large serotonergic pair of Retzius cells (RZ) and seven small interneurons are vitally and selectively stained by Neutral Red dye (Stuart, et. al., Cell Tiss. Res. 55:61, 1974), a procedure potentially facilitating intracellular investigations of a limited population of biochemically-related neurons.

Simultaneous recordings from RZ and each of the small MA cells show a pattern of slow depolarizations producing impulse bursts in temporal register. This pattern apparently results from the activity of a common excitor, and is also seen between pairs of small cells. Thus, the entire MA population receives an excitatory input. This central excitation is probably mediated by cholinergic synapses as the slow depolarizations are blocked by Mg^{++}, reversibly-abolished by atropine, and augmented by eserine.

RZ are coupled to one another by low resistance junctions and to each of the small MA cells by high resistance junctions. The high resistance junctions are Mg^{++}resistant, ohmic in the hyperpolarizing domain and rectify somewhat to depolarization. Similar high resistance junctions between the small cells suggest that the MA cell population is electrotonically-intercoupled.

Anodal break stimulation can produce self-sustaining bursts by the MA cell population. Thus, both patterns of connectivity (coupling and common excitation) assure a concerted impulse activity by the ganglionic MA cells. Furthermore, both types of burst activity are conducted along the ventral nerve cord of the leech generating bursts in adjacent ganglia. We infer from this that the central impulse activity of the monoamine neurons has an integrative function above the ganglionic level.

797 DEVELOPMENT OF THE NORADRENERGIC INNERVATION OF THE NEOCORTEX IN THE RAT. Pat Levitt* and Robert Y. Moore. Department of Neurosciences, School of Medicine, Univ. of Calif., San Diego, La Jolla, CA 92093.

The neocortex of the adult rat receives a diffuse, plexus-like noradrenergic innervation from the locus coeruleus which is identical in organization in all cortical areas. Fibers branch at all cortical levels to undergo extensive collateralization. The molecular layer contains the most dense fiber plexus as a result of terminal horizontal branching. This highly ramifying afferent system has been studied in terms of its role in the development of the neocortex. The present analysis concerns the time of origin and maturation of the noradrenergic fibers in the neocortex. The combined formaldehyde-glyoxylic acid freeze-dry histochemical technique and high affinity uptake of ^{3}H-noradrenaline into tissue slices were used for developmental analysis.

Fluorescence histochemistry reveals a sparse innervation of the frontal, occipital, and lateral cortices by 20 days gestation which is localized to the cortical plate. Innervation of the parietal cortex is not observed until postnatal day 1. Slow development proceeds in all cortical areas up to day 3, when noradrenaline-containing fibers are evident in all cortical layers. Typically, a sparse vertical plexus with few branches is observed in the deep layers, which ramifies into a much more dense horizontal plexus in the molecular layer. By day 6 the developing innervation resembles the adult, though a significant variation in fiber density is evident. At 10 days, the pattern and density look identical to the adult neocortex.

Accumulation of ^{3}H-noradrenaline by high affinity uptake reveals a similar pattern of development. On postnatal day 2 uptake is only 10% of adult values, but rapidly increases to nearly 90% by day 9. Uptake values reach approximately 2.5 times the adult between days 10 and 18. Thereafter, a gradual decrease to adult levels occurs. This suggests that the noradrenergic innervation is complete at an early postnatal stage, prior to the development of the cerebral neocortical neuropil. (Supported by USPHS NS-12080)

798 THE MONOAMINERGIC INNERVATION OF IMMATURE RAT CORTEX: AN EXPERIMENTAL HISTOFLUORESCENCE STUDY. Hart G.W. Lidov and Mark E. Molliver. Departments of Anatomy and Neurology, The Johns Hopkins School of Medicine, Baltimore, Md. 21205. U.S.A.

The monoaminergic (MA) projections from brainstem are among the first axons to invade the developing forebrain and may exert a trophic influence as well as function in neurotransmission. *Ultrastructural studies* of neonatal rat cerebral cortex, from this laboratory, have demonstrated many MA synapses in strata parallel to the pia. Prior efforts to visualize the presynaptic processes using *histofluorescent techniques* have been hampered by low MA levels in infancy. *Biochemical studies* have shown that monoamine neurons develop a MA uptake-storage mechanism in advance of transmitter synthesis, and that immature cortex has a MA storage capacity 10-fold greater than endogenous levels. We have taken advantage of these biochemical properties by treating with MA congeners and thus augmenting the low endogenous levels, in order to demonstrate the full extent of the MA innervation by light microscopic histofluorescence.

Cingulate and lateral neocortex from 6-day old rats was studied with glyoxylic acid induced histofluorescence. Treatment with an MAO inhibitor (nialamide, 100 mg/kg) favors visualization of those fibers with substantial endogenous MA stores; treatment with a MA congener (α-methylnorepinephrine, α-MNE, 75 mg/kg) displays the larger set of fibers with an active MA uptake mechanism. In a third group surgical or chemical lesions were made in the midbrain tegmentum as a control for uptake into non-aminergic axons.

The results are that 1) nialamide treated rats exhibit a sparse MA innervation of layer I; 2) there is a striking increase in the quantity of fluorescent elements after α-MNE; 3) the MA fibers are predominantly in three strata parallel to the pia: at 30μ, 75μ, and a broad peak at 250μ, subpial. These strata are coincident with the primordia of layers I and IV. One interpretation of our findings is that the three strata are the terminal fields of three distinct MA projections to immature cortex. The dense, latent MA innervation in layer IV of cortex corresponds to the major stratum of MA synapses demonstrated ultrastructurally and is the morphological expression of the MA storage compartment defined biochemically [Science 196 (1977) 444]. The existence of fibers which do not exhibit transmitter, but have been shown ultrastructurally to form synapses, suggests that synaptogenesis does occur in the absence of neurotransmitter. [Support: USPHS NS-08153, NS-10920 and United Cerebral Palsy Grant R244-71; H.G.W.L. supported by Training Grant GM-7309]

799 EFFECTS OF MANIPULATION OF CENTRAL SEROTONIN ON LEARNED TASTE AVERSIONS IN THE RAT. Joan F. Lorden and Gary A. Oltmans. Dept. Psych., University of Alabama in Birmingham, Birmingham, AL, 35294 and Dept. Pharm., Chicago Medical School, Chicago, IL 60612.

Depletion of central serotonin (5HT) in rats has been shown to facilitate avoidance training when painful electric footshock is used as a stimulus (Lorens et al., JCPP, 77:48, 1971). The experiments reported here examined the effects of 5HT depletion on another type of avoidance behavior, the conditioned taste aversion. Rats made sick following the ingestion of a novel substance will avoid that substance on subsequent presentations. In this paradigm, gastrointestinal distress or other drug effects, rather than cutaneous pain, serves as the unconditioned stimulus. Electrolytic lesions of the dorsal and median raphe nuclei in rats enhanced the avoidance of a saccharin solution that had been paired with a single injection of the toxic drug, lithium chloride (12 mg/kg, ip, of .15M LiCl). A chemically specific lesion of these nuclei made by infusion of 5,7-dihydroxytryptamine produced the same behavioral effect. Neither lesion group differed from control animals in the consumption of an unpaired familiar fluid or an unpaired novel fluid. Both lesions caused a significant depletion (66-68%) of telencephalic serotonin. The data suggested that the enhanced suppression of saccharin intake was due to damage to serotonergic neurons. Rats with electrolytic lesions of the median raphe alone showed a significant enhancement of saccharin avoidance in the taste aversion paradigm. The addition of a dorsal lesion increased 5HT depletion (71 vs 43%) but did not produce a significant increment in saccharin avoidance. Thus, decreased hippocampal or septal 5HT produced by the median lesion may account for the facilitation of the learned aversion.

The behavioral effects of the combined dorsal and median raphe lesions were reversed by pretreatment with DL-5-hydroxytryptophan (75 mg/kg, 5HTP) which has been shown to elevate forebrain 5HTP levels. In rats with dorsal and median raphe lesions, 5HTP administration blocked the formation of a learned taste aversion. In normal rats, attenuation of learned aversions was obtained with a lower dose of 5HTP (50mg/kg). Experiments using the flinch-jump technique to evaluate sensitivity to footshock indicated that treatment with 5HTP did not alter the flinch-jump thresholds of normal rats but did restore thresholds to normal in rats with lesions that depleted 5HT (Harvey & Lints, JCPP, 74:28, 1971). Thus, the conditioned aversion paradigm may be more sensitive than the flinch-jump technique to manipulation of of central 5HT levels.

(Supported in part by a UAB Faculty Research Grant)

800 INCREASED TILT-CAGE ACTIVITY FOLLOWING INTRACRANIAL ADMINISTRATION OF 5,7-DIHYDROXYTRYPTAMINE. Robert G. MacKenzie*, Charles Norelli*, Michael E. Trulson and Bartley G. Hoebel. Dept. of Psychol., Princeton Univ., Princeton, N.J. 08540

Previous work has shown that electrolytic lesions of the median raphe nucleus, a region rich in serotonin (5-HT)-containing cell bodies, are followed by increases in 24 hr home cage activity, wheel running, and open-field activity in rats. Synthesis inhibition of 5-HT by i.p. injections of p-chlorophenylalanine (PCPA) is also followed by increased locomotor activity; further suggesting serotonergic control over locomotion. However, recent studies have questioned such a role for 5-HT by demonstrating that depletion of forebrain 5-HT by the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) is either without effect or actually depresses locomotor activity in the open field.

This report demonstrates an increase in locomotor activity with 5,7-DHT injected either locally or intraventricularly. A 2-fold increase in 24 hr activity was measured by housing rats in tilt cages 3 weeks after administration of 5,7-DHT (8 µg) in the posterior medial forebrain bundle. This treatment caused a 79% depletion of forebrain 5-HT. The daily activity of the 5,7-DHT group remained elevated throughout the 36 consecutive day monitoring period. Intraventricular administration of 5,7-DHT (200 µg) resulted in an approximate 4-fold increase in 24 hr home cage activity, and 5-HT depletions of 83%, 58% and 71% in forebrain, brainstem, and spinal cord, respectively. These rats were housed in tilt cages one month following injection and activity was monitored for 14 consecutive days. The diurnal index of activity, computed as light activity/light & dark activity x 100 was identical for the 5,7-DHT group and controls, suggesting preservation of the normal light-dark activity rhythm which contrasts with the disruption of this rhythm reported in PCPA-induced hyperactivity. Furthermore, unlike rats with median raphe lesions, the behavior of the rats administered 5,7-DHT intraventricularly was undistinguishable from that of controls when tested in the open-field. This result shows that effects obtained from open-field activity cannot be generalized to other measures of locomotion.

It is concluded, in agreement with other reports, that different methods of 5-HT depletion can result in different effects on various behavioral measures, but that this does not appear to be the case for tilt cage hyperactivity. That is, this effect occurs following electrolytic raphe lesions, PCPA or 5,7-DHT. It is, therefore, suggested that this effect is not the result of non-specific damage but rather is due to subnormal 5-HT synaptic activity in the central nervous system.

801 INACTIVATION OF BRAIN TYROSINE HYDROXYLASE BY CYCLOHEXIMIDE IN VIVO. Keith Markey* and Paul Y. Sze. Dept. Biobehavioral Sci., Univ. of Conn., Storrs, CT 06268.

Following an injection of cycloheximide (300 mg/kg, s.c.) in mice, brain tyrosine hydroxylase (TH) activity was rapidly reduced. The decrease began to occur after 1 hr, reaching a minimum of 55-65% of control at 2.5 hrs and recovering to normal by 3.5 hrs. This decrease was observed in whole brain as well as regionally in pons, cerebral cortex and striatum, and also subcellularly in striatal synaptosomes. Kinetic analyses revealed that the apparent K_m of the enzyme for 6-MPH_4 was increased by 2-3 fold after cycloheximide, whereas the K_m for tyrosine remained unchanged. Addition of cycloheximide in vitro had no effect on TH activity. The alteration of K_m, the time course of the action, and the effect in nerve terminals indicate that the decrease of enzyme activity is not due to inhibition of protein synthesis by cycloheximide, directly or indirectly.

The characteristics of this inactivation were examined as follows: (1) Lower enzyme activity could be seen in 40,000 x g supernatant prepared with or without detergents (Triton X-100 or Lubrol), indicating that the effect is not altered by solubilization of enzyme activity. (2) TH from treated animals could be activated but not to normal levels by c-AMP, ATP, and Mg^{++}, or by heparin. (3) Mixing supernatants from treated and control animals yielded mean activity, suggesting the absence of a soluble modifier. (4) Mixing homogenates before centrifugation, however, fully restored the reduced activity to normal. It appears that a factor associated with some particulate material is involved here in modifying the TH activity.

Additional experiments using cycloheximide as a tool are underway to further characterize this inactivation of brain TH.

(Supported by MH 29237).

802 THE DORSAL NORADRENERGIC BUNDLE AND FRUSTRATIVE NON-REWARD. Stephen T. Mason* & Susan D. Iversen* (SPON: H.C. Fibiger). Psychological Laboratory, University of Cambridge, England.

Destruction of the dorsal noradrenergic bundle using 6-hydroxydopamine (6-OHDA) produces a resistance to extinction in a number of behavioral tasks. One mechanism by which this might occur would be an inability to code for non-reward or an alteration in the frustration produced by omission of an expected reward. This is tested by examining the response invigorating effects produced by randomly omitting some of the food deliveries on a fixed interval (FI) schedule. Response rate in the interval following that in which reward was omitted is known to increase in normal rats and thus provide a measure of the invigorating effects of frustration. Male albino rats weighing 200 gms were stereotaxically injected with 8 µg of 6-OHDA base dissolved in 2 µlitres of 0.9% saline with 1 mg/ml ascorbic acid antioxidant infused over 2 mins bilaterally at coordinates - 6mm from bregma, 0.8 mm lateral from midline and 5 mm below dura according to Konig & Klippel. After behavioral testing a biochemical assay of brain regions of these same animals confirmed that they had sustained depletions of cortical noradrenaline (NA) to 4% of controls, hippocampal NA to 11% while sparing cerebellar and hypothalamic NA and brain dopamine. Following the operation the animals were food deprived to 90% of their free-feeding weight and trained for 16 days on a FI 60 sec schedule of 20 FIs per day. No difference was found in acquisition between treated and control rats. When FI performance had stabilized reward was randomly omitted from ¼ of the intervals and the response rate in the following interval compared with that in intervals following presentation of reward. Omission of an expected reward increased the response rate significantly ($p<0.05$) but did so equally for both treated and control animals ($t=0.19$, $df=12$, NS). However, following 10 days of training on this schedule the treated and control rats diverged; with the controls showing a decreased frustration following reward omission but the treated rats continued at the same high initial level ($p<0.01$). Both groups were then retained on a 100% reinforced FI schedule to the same level of responding and extinguished in the complete absence of reward. The treated animals were resistant to extinction showing greater responding ($p= 0.042$) and taking longer to reach an extinction criterion ($p<0.05$). Thus, both biochemical and behavioral measures confirm the validity of the lesion yet it failed to alter the immediate response invigorating effect of reward omission, suggesting that these animals can code adequately for non-reward, but the lesion did prevent the decrease in frustration over time seen in the controls suggesting that the treated animals may be impaired in the memory of or the learning about non-reward.

803 THE EFFECT OF LESIONS OF THE VENTRAL NORADRENERGIC BUNDLE ON SEROTONIN AND NOREPINEPHRINE CONCENTRATION IN DISCRETE NUCLEI. V. John Massari*, Y. Tizabi* and D. M. Jacobowitz (SPON: Alan M. Laties). Lab. Clin. Sci., NIMH, Bethesda, MD 20014; and Dept. Pharmacology, Howard Univ., Washington, D.C. 20059.

Since fluorescence-histochemical studies first noted the close apposition of catecholaminergic (CA) nerve terminals to serotonergic (5HT) cell bodies, increasing evidence has accumulated to suggest that the functional activity of 5HT neurons may be regulated by CA. CA and their synthesizing enzymes have been measured in each of the raphe nuclei (Brain Res. 114: 339, 1976), and drugs which effect CA, such as 6-hydroxydopamine or α-methyl-p-tyrosine, have also been shown to effect 5HT metabolism. Similarly, 5HT neurons have been shown to be capable of influencing NE metabolism. Very little information is available, however, to define specifically which 5HT and noradrenergic (NE) nuclear groups and axon pathways are involved in this interaction. In this study we have severed the caudal aspect of the ventral NE bundle and thereby significantly reduced the A-1 and A-2 cell body contributions to this projection. Subsequent effects on 5HT content in discrete nuclei were examined. Male Sprague-Dawley rats received bilateral knife cut lesions of the ventral NE bundle (caudal to the locus coeruleus). Controls received a sham lesion into the cerebellum. Two weeks later all animals were sacrificed by decapitation and a 3 mm slice of the medulla was taken for histofluorometric confirmation of the accuracy of the lesions. The rest of the brain was processed by the microdissection method of Palkovits. The ventral NE bundle lesions did not effect levels of NE in the locus coeruleus or in two or its terminal projection areas (cingulate cortex, habenula). Similarly, 5HT levels in the dorsal raphe nucleus, interpeduncular nucleus, substantia nigra reticulata, area tegmenti ventralis, lateral mammilary nucleus, median forebrain bundle, retrochiasmatic area, hippocampus, and striatum were also uneffected. However, 5HT levels in the median raphe nucleus were significantly reduced by 40%. These results suggest that, unlike the previously proposed epinephrine projection, there does not appear to be a substantial noradrenergic pathway to the locus coeruleus from the A-1 and A-2 cell groups. This study provides support for the hypothesis that a NE projection to the median raphe nucleus may modulate 5HT neuronal function, although it cannot be excluded that destruction of some other unknown fiber system traveling with the ventral NE bundle may be responsible for the observed effects.

804 EXCESSIVE ELEVATION OF PLASMA CATECHOLAMINES DURING STRESS IN SPONTANEOUSLY HYPERTENSIVE RATS. R. McCarty and I.J. Kopin, Laboratory of Clinical Science, NIMH, Bethesda, MD 20014.

Recent evidence suggests an increased activity of the sympatho-adrenal system of spontaneously hypertensive rats (SHR) when compared to a matched normotensive strain (WKY). This difference is most pronounced in young SHR prior to the elevation in blood pressure (Grobecker et al., Nature, 258: 267, 1975; Nagaoka and Lovenberg, Life Sci., 19: 29, 1976; Nagatsu et al., Science, 191: 290, 1976). We were interested in measuring the responsiveness of the sympatho-adrenal system to acute stress in SHR and WKY rats.

Rats of each strain were obtained at 6,18 and 48 weeks of age. A chronic tail arterial catheter was inserted into each rat while under pentothal anesthesia. Two days after surgery, each rat was transferred from its home cage to a shock chamber and after 5 min received 60 footshocks (2.5mA, 0.4 sec duration) over a 5 min interval. Blood samples were taken from undisturbed animals in the home cage, 3-5 min after transfer to the shock chamber, and at the end of footshock. Plasma levels of norepinephrine (NE) and epinephrine (EPI) were measured by a sensitive radioenzymatic assay.

There were no strain differences in plasma levels of NE or EPI while animals were at rest in the home cage. Transfer from the home cage to the shock chamber resulted in a greater increase in plasma levels of both catecholamines in SHR rats of each age group. A similar pattern was evident after footshock; SHR rats had significantly higher post-shock levels of plasma NE and EPI when compared to age-matched WKY rats. These results demonstrate that the sympatho-adrenal system of SHR rats is more responsive than normotensive rats to stressful stimuli and that this hyperresponsivity is independent of increases in blood pressure. The excessive discharge of EPI and NE into plasma during stress may contribute to the development and maintenance of high blood pressure in SHR rats.

805 LOCOMOTOR BEHAVIOR INITIATED BY THE MICROINJECTIONS OF PICROTOXIN INTO THE VENTRAL TEGMENTAL AREA. G. J. Mogenson, M. Wu* and S. K. Manchanda*. Departments of Physiology & Psychology, University of Western Ontario, London, Canada.

Microinjections of dopamine, L-DOPA or apomorphine, a dopamine agonist, into the nucleus accumbens have been shown to initiate locomotor behavior in rats (Pijnenberg & Von Rossum, J. Pharm. Pharmacol., 1973, 25, 1003-1005; Costall & Naylor, Europ. J. Pharmacol., 1975, 32, 87-92). These compounds are assumed to act on post-synaptic receptors in the nucleus accumbens of dopaminergic (A_{10}) neurons which project from the ventral tegmental area (VTA). Since dopaminergic neurons receive GABA synaptic inputs exerting inhibitory effects (Füxe et al, Med. Biol., 1975, 53, 177-183) an investigation was carried out to see whether blocking these GABA inputs with picrotoxin, a GABA antagonist, would also initiate locomotor behavior. Following bilateral microinjections of picrotoxin (0.10 μg in 0.2 μl) into the VTA of rats the locomotor activity, as indicated by the number of counts in a photocell activity chamber, increased from a mean value of 47 to 134.8 ($p < 0.05$). Increased locomotion was accompanied by decreased grooming responses. These changes in locomotion were dose-dependent when tested with graded doses of picrotoxin (0.05 - 0.2 μg). Locomotor activity was also increased by unilateral microinjections of picrotoxin into the VTA but the magnitude of the response was significantly less. Control injections of strychnine sulphate (0.10 - 0.15 μg in 0.2 μl) or isotonic saline did not increase locomotion. Bilateral microinjections of spiroperidol (1.5 μg in 0.2 μl), a dopamine antagonist, into the nucleus accumbens significantly reduced the locomotor activity induced by picrotoxin (0.15 μg in 0.2 μl) into the VTA from a mean value of 235.6 to 90.6 ($p < 0.05$). The bilateral application of picrotoxin (0.15 μg in 0.2 μl) to the substantia nigra also increased locomotor activity but this increase was less than observed with VTA microinjections. Unilateral infusions of picrotoxin into the substantia nigra were followed by marked rotational behavior to the side contralateral to the stimulation site. These results are consistent with a role for dopaminergic (A_{10}) neurons of the VTA in initiating locomotor activity via projections to the nucleus accumbens. (Supported by the Medical Research Council of Canada and the National Research Council of Canada).

806 LOCUS COERULEUS STIMULATION POTENTIATES PURKINJE CELL RESPONSES TO IONTOPHORETICALLY APPLIED GAMMA AMINOBUTYRIC ACID. Hylan C. Moises and Donald J. Woodward. Dept. Cell Bio., Univ. Tx. Health Sci. Ctr., Southwestern Med. Sch., Dallas, Tx. 75235

We have recently reported that local administration of norepinephrine (NE) can selectively enhance excitatory and inhibitory responses of Purkinje (P) neurons produced both synaptically and by microiontophoretic application of putative cerebellar amino acid neurotransmitters, notably glutamate and gamma aminobutyric acid (GABA). In this study we asked whether NE, released synaptically during activation of the noradrenergic pathway from locus coeruleus (LC) to cerebellum, can modulate P cell responses to iontophoretically applied GABA.

Multibarrel micropipettes were used to apply drugs and to record P cells extracellularly in 20 Halothane-anesthetized rats. Post-stimulus time histograms were used to assess unit activity influenced by electrical stimulation in the region of LC. Histograms were also constructed to quantitate the effects of microiontophoretic pulses (10 sec. duration at 30 sec. intervals) of GABA (15-30 nA) applied before, immediately after (2.5-5 sec.) and during recovery from (60 sec. after) sub-threshold LC stimulation. Results showed in 12 of the 30 neurons studied, that brain stimulation at sites outside LC (brachium conjunctivum, juxtafastigial white matter) produced only transient effects on activity consisting of short latency (2-7 msec.) excitations, followed immediately by brief inhibitory periods (10-150 msec.). Repetitive stimulation in areas outside LC did not alter the inhibitory effects of GABA on any of the 8 cells tested from this group.

Eighteen P cells showed prolonged slowing or cessation of spontaneous discharge to discrete stimulation of LC with trains of shocks (20-100 pulses of 0.1-1.0mA delivered at 10/sec.). This response, which outlasted the stimulation by 3 to 30 seconds, has been attributed to synaptic release of NE. The effects of LC stimulation on inhibitory responses to iontophoretically applied GABA were examined in 6 of these cells. In all 6 cells, the inhibitory effects of GABA were potentiated by short preconditioning volleys (10/sec. for 2.5-5.0 sec.) delivered to LC 7.5 seconds before each GABA pulse. In 4 cells, augmentation of GABA inhibition was observed at stimulation currents which alone elicited no depression of spontaneous discharge. These data provide additional evidence that activation of the noradrenergic pathway from LC to cerebellum acts to modulate Purkinje cell responsiveness to putative cerebellar neurotransmitters. (Supported by NIH Grant NS 13225 and NSF Grant GB 43301 to D.J.W.).

807 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF NORADRENERGIC INNERVATION IN THE RAT NEOCORTEX: A REGIONAL AND LAMINAR ANALYSIS.
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Based on catecholamine histofluorescence, the noradrenergic (NA) innervation of the rat neocortex has been described as sparse, with the highest density of fibres in layer I. An alternative method for the identification of noradrenergic neurons makes use of dopamine-β-hydroxylase (DBH) as a specific antigenic marker. Using an antiserum prepared against rat DBH[1] (which has a 10^4 greater affinity for the rat enzyme than does heterologous antiserum) we have identified an unexpectedly dense NA innervation throughout rat neocortex[2].

The NA fibre distribution in 4 cytoarchitectonic regions of cortex (cingulate, somatosensory, motor and visual) was examined in sagittal and coronal sections prepared from over 100 rats. Cryostat or Vibratome sections were incubated with anti rat DBH and then processed for the immunofluorescence technique or by Sternberger's unlabeled antibody technique (PAP). Photomontages through all of the 4 cortical areas were analyzed.

In each of the 4 cortical regions examined, a dense NA innervation was found with a characteristic pattern in each layer. In layer I of somatosensory cortex numerous long axons run parallel to the pial surface predominantly in a coronal plane. In contrast, layers II and III contain long, radially oriented axons. Layer IV is strikingly different in that it contains numerous short, oblique axon segments. No radial or tangential axons traverse layer IV. Layers V and VI contain a high density of obliquely oriented short as well as long axon segments; in addition, layer VI contains many long tangential axons running in the coronal plane. This general pattern of NA innervation was found in all areas of neocortex with some regional variations in the 4 inspected areas. We have observed many NA axons making contact with cell bodies and proximal dendrites in layers IV to VI (using bright-field and Nomarski optics with PAP stained material); these data reveal a direct NA innervation of cell bodies in deep cortical layers. The demonstration of long, tangential fibres in layers I and VI provide an anatomical substrate by which individual NA axons can influence adjacent cortical columns over long distances in the coronal plane. (Support: USPHS NS-08153, NS-10920, MH-26654, UCP and Deutsche Forschungsgemeinschaft GR 504)

[1] Grzanna, R. and Coyle, J. T., J. Neurochem. 27 (1976) 1091
[2] Grzanna, R., Morrison, J. H., Coyle, J. T. and Molliver, M. E. Neurosci. Lett. 4 (1977) 127

808 A NEW ROLE FOR THE LOCUS COERULEUS.
A.R. Morrison, J.C. Hendricks‡ and R.M. Bowker*, School of Vet. Med., U. of Pa., Phila., Pa.

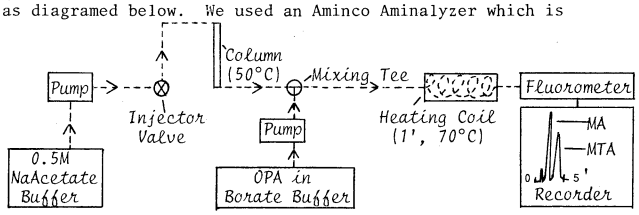
Paradoxical sleep (PS) without atonia is a dramatic phenomenon created by small bilateral lesions in the pons in cats and is characterized as follows: After slow wave sleep, when PS would normally appear, cats raise their heads, make body righting movements, exhibit alternating movements of the limbs, and even attempt to stand. Throughout an episode, which shows all other aspects of PS, cats act as if they are being startled, searching or even attacking an object. During wakefulness they act aggressively. Jouvet maintains that this phenomenon, which supplants normal PS, results from damage of the caudal locus coeruleus (LC); but we have argued that more ventral pontine damage is more critical. Additional results suggest that the complete behavioral syndrome of PS without atonia depends upon interruption of inputs into LC from the pontine tegmentum and relative integrity of LC itself.

We suggest an explanation for PS without atonia drawn from data from the locomotion literature. Decerebrate cats will walk on a treadmill if stimulated electrically in the region of the ponto-mesencephalic isthmus, the "mesencephalic locomotor region". The most effective stimulus sites are adjacent to noradrenergic LC neurons. Activity of this inhibitory noradrenergic system permits movement by releasing an intraspinal neuronal assembly which activates extensor and flexor motor neurons bilaterally in a reciprocal manner. L-DOPA, which releases noradrenalin from spinal terminals, has the same effect.

We propose that pontine lesions interrupt fibers which inhibit LC neurons during PS. They also presumably impair cholinergic mechanisms facilitating atonia. Thus the cats are freed to express elements of locomotor activity. Rather than a combination of electrical stimulation and a treadmill, the stimulus source would be intrinsic, i.e. the neural activity of PS which initially produces"alerting" and "orienting" in these cats prior to "searching" with varying degrees of success at locomotion. Their behavior is more variable than that of the decerebrate locomotor preparation because di- and telencephalic structures can elaborate upon patterns generated in the brainstem; and the cats are free-moving, not on a treadmill. Furthermore, the fact that pontine giant neurons fire during wakefulness if cats are free to move, as well as during PS, suggests that a pontine mechanism designed to act beyond the confines of PS has been revealed. Its role is modulation of movements, especially those generated by novel alerting or startling stimuli of any modality which require immediate control and dampening within the reticular formation lest the animal overreact and run blindly into danger prior to analyzing the stimulus source and significance. Supported by NIH grants RR07083-11,GM02051 andMH15767

809 MEASUREMENT OF THE FALSE TRANSMITTERS, METARAMINOL AND α-METHYL-m-TYRAMINE, IN RAT BRAIN BY HIGH PRESSURE LIQUID CHROMATOGRAPHY: AN INDEX OF NOREPINEPHRINE AND DOPAMINE TURNOVER.
K. W. Perry* and R. W. Fuller. The Lilly Res. Labs., Indianapolis, IN 46206.

The "false transmitters" metaraminol (MA) and α-methyl-m-tyramine (MTA) are formed after injection of α-methyl-m-tyrosine (100 mg/kg i.p.) and are stored in norepinephrine and dopamine neurons, respectively [Dorris and Shore, J. Pharmacol. exp. Ther. 179, 10 (1971)]. The rate of decline of these false transmitters can then be used as an index of norepinephrine or dopamine turnover. We have developed a high pressure liquid chromatography (HPLC) assay for MA and MTA that greatly facilitates their measurement in rat brain. The rat brain (or brain region) is homogenized in 0.4 M $HClO_4$; after centrifugation the supernatant is adjusted to pH 9 with 5 N NaOH. MA and MTA are extracted into ethyl acetate and after evaporation of the ethyl acetate are dissolved in 0.2 ml 0.01 N HCl for injection onto a pellicular cation exchange column (Vydac, 2 mm x 50 cm). The column effluent flows (0.5 ml/min) to a mixing tee where it is mixed with o-phthalaldehyde (OPA, 800 mg/l) in 0.5 M sodium borate buffer pH 10 (flow rate = 0.25 ml/min) then to a heating coil and subsequently to the fluorometer (360 nm excitation, 480 nm emission), as diagramed below. We used an Aminco Aminalyzer which is



specifically designed for HPLC with fluorescence analysis after OPA reaction, however a less expensive instrument could be assembled by the user. Under these conditions the OPA reaction is specific for MA and MTA [Shore and Alpers, Life Sci. 5, 551 (1964)], which are completely resolved by the column, and the maximum sensitivity is about 1 ng for both compounds. We investigated the utility of MA and MTA as an index of turnover by measuring the decline in MA after injection of prazosin, a norepinephrine α receptor antagonist, and the decline in MTA after lergotrile, a dopamine receptor agonist. Prazosin increased the decline in MA, which is consistent with the increased turnover in norepinephrine that a receptor antagonist is expected to produce. Lergotrile decreased the decline in MTA as expected of a dopamine agonist, but it also increased the decline in MA indicating that lergotrile may also be an α receptor antagonist.

810 EFFECTS OF 5-HT DEPLETION ON RAT HOLE-BOARD EXPLORATION: DEPENDENCY UPON METHOD.
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In this series of experiments we studied the influence of the serotonergic system on exploratory nose-poking behavior measured in a hole board. The hole board consisted of a rectangular wooden box (11 x 26 in) with its walls extending below the level of the floor. A nose-poke was scored when a rat dipped its head into one of three holes spaced equally on the midline of the floor. For the duration of each nose-poke a dim light was lit to provide a visual feedback stimulus to the rat. Both the response frequency and mean duration per response (MDPR) were cumulated during each 8 min of a 24-min test period.

Selective electrolytic lesions were made either to the dorsal or median raphe nuclei, which are the origins of the mesostriatal and mesolimbic serotonergic pathways respectively. Median raphe-lesioned rats exhibited a significantly higher response frequency ($p < 0.001$) and MDPR ($p < 0.001$) than sham-lesioned controls. Dorsal raphe lesions produced no significant effect on hole-board behavior.

In another group of animals chemical lesions were made by direct infusion of 5,7-dihydroxytryptamine (5,7-DHT) into the median raphe nucleus (0.25 µl; 10 µg/µl), the hippocampus (bilaterally, each side 5.0 µl; 5 µg/µl), or the lateral ventricle (10 µl; 10 µg/µl). 5,7-DHT is a cytotoxin having preferential effects on serotonergic neurons. To preclude damage to noradrenergic neurons, all animals were pretreated with the catecholamine-uptake inhibitor, desmethylimipramine. 5,7-DHT failed to produce a significant increase in either response frequency or MDPR. These results suggest that the increase in response frequency and MDPR with electrolytic lesions may be due to non-specific damage of fibers passing near the median raphe nucleus. To further examine the relevance of brain serotonin depletion to the effects of median raphe lesions on hole-board behavior, p-chlorophenylalanine (PCPA), a specific inhibitor of serotonin biosynthesis, was administered, 300 mg/kg i.p. 24 and 48 hrs before testing in the hole board. No significant effect on response frequency was observed.

811 IMMUNOCYTOCHEMICAL LOCALIZATION OF SUBSTANCE P CONTAINING AXON TERMINALS IN RELATION TO CATECHOLAMINE CONTAINING NEURONS IN RAT BRAIN. V.M. Pickel, T.H. Joh, D.J. Reis, and S.E. Leeman. Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021, and Dept. of Physiol., Harvard Medical School, Boston, MA 02115.

The polypeptide, substance P is present in relatively high concentrations in areas containing dopamine neurons (Brownstein et. al., Brain Res 116.299, 1976). We sought to examine the interaction between substance P and catecholamine neurons, both dopaminergic and noradrenergic, by the light and electron microscopic immunocytochemical localization of antisera to substance P and to tyrosine hydroxylase (TH), the enzyme which catalyzes the first step in catecholamine biosynthesis. Adjacent sections of rat brainstem were incubated with either TH antiserum, substance P antiserum, or normal rabbit serum. These sections were then labeled by the peroxidase-antiperoxidase (PAP) technique and processed for light and electron microscopy. By light microscopy, sections incubated with TH antiserum contained labeled neuronal perikarya in nuclear groups containing catecholaminergic neurons (A1-A14) as classified by Dahlstrom and Fuxe (Acta Physiol Scand 62: Suppl, 232, 1964). Adjacent sections incubated with antiserum to substance P contained a dense plexus of labeled axonal varicosities in the areas labeled for TH perikarya. Control sections were devoid of labeled perikarya or processes. By electron microscopy, the specific labeling for TH and substance P was examined only in the A1 and A6 (locus coeruleus) nuclear groups. TH was localized to the cytoplasm of catecholaminergic neurons, particularly in association with the endoplasmic reticulum and microtubules. Substance P was present in axons and in axon terminals. The labeling for substance P was localized in the axon terminals primarily in association with large (80-100 nm) dense cored vesicles, however moderate reaction product was also associated with small clear vesicles. These labeled terminals were in close apposition to dendrites of the catecholamine containing neurons. These findings suggest that the activity of central noradrenergic and dopaminergic neurons may be regulated by neuronal systems which contain substance P.

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812 MICROSPECTROFLUOROMETRIC DETERMINATION OF DOPAMINE IN MIDBRAIN CELL AGGREGATES. George M. Powell*, Robert J. Dinerstein* and Beatrice Garber. Dept. Pharm. and Physiol. Sci. and Dept. Biol., Univ. of Chicago, Chicago, Ill. 60637

It has been shown previously (Levitt, Moore and Garber, 1976) that fluorescent cells derived from 14-18 day embryonic mouse mid-brain containing the substantia nigra can be recovered in reaggregated tissues after complete dissociation into single cell suspensions and rotary culture in vitro. Using the Falck-Hillarp histofluorescence method it was found that after 4 or more days in culture the fluorescent cells had selectively associated and extended varicosities into the surrounding tissue. The assay of Coyle and Henry (1973) employing enzymatic conversion of catecholamines to radiolabeled O-methylated derivatives showed that dopamine is the principle catecholamine in these aggregates. Since this biochemical demonstration was indirect evidence for identifying these fluorescent cells as dopaminergic neurons, we have examined these cells by microspectrofluorometry. We have now shown unequivocally by direct measurements that the selectively associated fluorescent cells in these aggregates contain dopamine. The anterior mesencephalic ventral tegmentum was dissected according to a consistent procedure so that dopamine cell groups A9 and A10 (Dahlstrom and Fuxe, 1965) were obtained and other monoamine groups were excluded. The mesencephalon was dissected out from a point just caudal to the pineal primordium to a point in the middle of the presumptive inferior colliculus, then the caudal tegmentum, the tectum, and adhering diencephalon (when present) were removed. Cell aggregates cultured for 5-12 days were harvested after treatment with pargyline HCl (0.375 mg/ml) for 12 hours, and processed by standard Falck-Hillarp techniques using paraformaldehyde equilibrated at 50% RH. Spectra confirmed that the fluorescent cells contained catecholamine with a 410 nm excitation maximum and a 480 nm emission maximum. To distinguish dopamine from norepinephrine the method of Bjorklund, Ehringer and Falck (1972) was used. Sections were partially deparaffinized with xylene and were repeatedly exposed to HCl-vapor over a 15 minute period. Control embryonic tissue in situ showed that with increasing times of exposure to HCl-vapor, norepinephrine in the choroid plexus showed a progressive decline in the 370 nm/320 nm peak intensity ratios of the excitation spectrum. Under the same conditions, dopamine in the substantia nigra of the same embryonic tissue showed no change in excitation spectrum. The catecholamine containing cells of the experimental aggregates showed no 370 nm/320 nm ratio changes under the same conditions, thus confirming the dopaminergic identity of selectively associated histofluorescent cell groups as substantia nigra and ventral tegmental dopaminergic neurons.(PHS NS12324,MH14274,NS10714)

813 COMPARISON OF THE ANTI-APOMORPHINE ACTIVITY OF LENPERONE AND HALOPERIDOL IN RABBITS AND RATS. R. M. Quock and R. A. Louie*. Dept. Physiol-Pharmacol., School of Pharmacy, Univ. of the Pacific, Stockton CA 95211.

Lenperone [4'-fluoro-4-(4-fluorobenzoyl)piperidinyl butyrophenone hydrochloride] is a new antipsychotic compound structurally related to haloperidol and found to be effective in acute schizophrenia (Harris, J. Clin. Pharmacol. 15, 187, 1975). An advantage of this drug over haloperidol is its relatively low incidence of extrapyramidal syndrome (EPS). Since EPS is thought to originate from an action of the drug upon the corpus striatum (Anden, J. Pharm. Pharmacol. 24, 905, 1972), we examined and compared the interactions of lenperone and haloperidol with the dopaminergic agonist apomorphine in rabbits and rats.

In male New Zealand rabbits, haloperidol (0.5 mg/kg iv) abolished the hyperthermic, locomotor stimulatory and stereotypic gnawing responses to apomorphine (5.0 mg/kg iv). At 0.5 mg/kg and even higher doses, lenperone antagonized only the temperature and locomotor responses to apomorphine. In male Wistar rats, haloperidol (0.3 mg/kg ip) blocked the hypothermic, locomotor stimulatory and stereotypic gnawing responses to apomorphine (2.0 mg/kg ip). At the same dose, lenperone antagonized the temperature and locomotor effects; the intensity of stereotypic gnawing was unaltered but the duration of the gnawing response was shortened.

In conclusion, our data indicate that lenperone differs from haloperidol in its spectrum of anti-apomorphine activity in both rabbits and rats. While haloperidol nonselectively antagonized all three apomorphine responses, lenperone selectively blocked the temperature and locomotor responses and spared the gnawing response. Since stereotypic gnawing is thought to originate in the corpus striatum (Ernst and Smelik, Experientia 22, 837, 1966), our data suggest that lenperone possesses greater selectivity of action than does haloperidol. (Supported in part by a Research Starter Grant from the P.M.A. Foundation.)

814 MODULATION OF THE UNITARY ACTIVITY OF CORTICAL NEURONS BY THE BIOGENIC AMINES: A MICROIONTOPHORETIC STUDY. Tomas A. Reader, André Ferron* and Laurent Descarries. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

In order to investigate the mode of action of biogenic amines in relation to cortical activity, the effects of microiontophoretic applications of dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) on single cell responses were examined in the fronto-parietal neocortex of adult rats. Unitary activity was assessed by recording the pattern and frequency of discharges before, during and after administration of these compounds (ejection currents: 50-100 nAmp; duration of ejections: 15-30 s). DA, NA and 5-HT were found to inhibit the spontaneous or glutamate-induced firing of a majority of the tested neurons. In the case of spontaneously active units, this inhibition was usually of prolonged duration (4-6 min). It was less abrupt in onset and of lesser magnitude with DA than NA or 5-HT. In glutamate activated cells (40 nAmp, during 8 s at every 20 s), the inhibition intervened more rapidly, persisted throughout the duration of monoamine ejection and ceased sooner thereafter. The interaction between DA, NA, 5-HT and acetylcholine (ACh) was studied on cholinoceptive units showing or not spontaneous activity. In both instances, DA and NA slightly reduced the excitatory response to ACh (45 nAmp during 5 or 10 s at every 90 s), in terms of speed of onset, magnitude, and especially of duration. It was further observed that the inhibition of the ACh response by DA or NA was less prolonged than that produced by these compounds on the spontaneous activity of the same cells. Conversely, the temporal course of DA or NA induced inhibition was shortened when ACh was applied. Under the influence of 5-HT, the excitatory responses to ACh were completely blocked. The return to initial levels of activity was slow and progressive, and the total duration of the inhibitory effect was not shorter than the inhibition induced by 5-HT on the spontaneous activity of the same cells. Moreover, the inhibition by 5-HT of spontaneous cell firing was not modified by ACh. These results are compatible with a modulatory role for biogenic amines in the cerebral cortex and demonstrate an interaction between these compounds and acetylcholine.

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815 ANXIETY: THE LOCUS COERULEUS CONNECTION. D.E. Redmond, Jr., Y.H. Huang, and M.S. Gold. Dept. Psychiatry, Yale Univ., New Haven, Ct. 06510

Specific behaviors elicited (1) by "dangerous" situations, (2) by a drug which activates the locus coeruleus (piperoxane), or (3) by weak electrical stimulation of the locus coeruleus (LC) or dorsal noradrenergic bundle (DB) are blocked by agents with demonstrated anti-anxiety effects in humans: (1) diazepam and (2) propranolol. Both of these agents, and other known anti-anxiety agents to be discussed, can be linked to effects on the locus coeruleus and empirically block the behavioral effects of electrical stimulation of the locus coeruleus. Propranolol blocks post-synaptic ß-adrenergic projections from the locus coeruleus (Hoffer et al, BR. RES. 25:523, 1971; Segal and Bloom, BR. RES. 72: 99, 1974). Diazepam presumably blocks locus coeruleus activity by way of the inhibitory γ-amino-butyric acid (GABA) system (Keller et al, NAUN.-SCHM.ARCH.PHARM. 294: 1, 1976; Dray and Straghan, J.PHARM.PHARMAC. 28: 314, 1976) and the GABA receptors on the cell bodies of the LC (Iversen and Schon, NEW CONCEPTS IN TRANSMITTER REGULATION, Plenum, p. 153, 1973) which inhibit its firing (Cedarbaum and Aghajanian, BR. RES. 112: 413, 1976). Piperoxane which increases the same behaviors associated with threats or following electrical stimulation of the LC has been reported to cause anxiety in humans (Goldenberg et al, JAMA 135: 971, 1947; Soffer, MED. CLIN.N.AMER. 38: 375, 1954. Electrical stimulation in the region of the LC in humans has been reported to produce feelings of fear and death (Nashold et al, ADV. NEUROL. 4: 191, 1974). These data support our previous suggestion (Redmond et al, BR. RES. 116: 502, 1976) that the nucleus locus coeruleus may be a brain "alarm" system that is related to the human emotions of fear or anxiety. Further anatomical and neurophysiologic evidence for an essential role for the locus coeruleus in such an "alarm" system will be presented and some objections to this interpretation of the data will be discussed.

816 INDUCTION AND DELAYED ACTIVATION OF TYROSINE HYDROXYLASE IN NORADRENERGIC NEURONS OF A1 AND A2 GROUPS IN MEDULLA OBLONGATA OF RAT. B. Renaud*, T.H. Joh, D.W. Snyder, and D.J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021.

In the medulla oblongata, two groups of noradrenergic neurons are of importance in regulating the arterial pressure. the A1 group in the area of the lateralis reticularis nucleus which innervates the intermediolateral columns, the locus of preganglionic vasomotor neurons, and the A2 group, in the area of the nucleus commissuralis which innervates the nucleus tractus solitarii (NTS), a site of termination of baroreceptor afferents. We have previously demonstrated that in the pontine noradrenergic nucleus locus coeruleus (LC), prolonged elevation in the activity of the catecholamine synthesizing enzyme tyrosine hydroxylase (TH) can be produced by two distinct mechanisms: the first, induction, elicited by reserpine, is due to production of more enzyme protein (J Pharmacol Exp Ther 193:775, 1975). The second, delayed activation, elicited by the cholinergic agonist oxotremorine, results from an increase in catalytic activity without change in enzyme protein (J Pharmacol Exp Ther 200:523, 1977). In this study we sought to establish if TH in the A1 and A2 neurons of rat brain share common biochemical properties with those of the LC. Rats were treated with reserpine (10 mg/kg s.c.), oxotremorine (1.5 mg/kg s.c.), or vehicle and killed at various days thereafter. The A1, A2, NTS, and LC were removed by microdissection. A single injection of reserpine increased TH activity in A1, A2, and LC by 50, 30, and 300% respectively (P<.01). The onset was seen at 24 h, reached maximal levels at 3 d, and disappeared by 21 d. In NTS a significant (P<.01) 30% increase of TH occurred but was delayed by 24 h after that of A2, suggesting slow transport of enzyme or activator from cell bodies into terminals in NTS. By immunotitration with an antibody to TH the increase of TH in A1 was demonstrated as a consequence of more enzyme protein and hence represented induction. Oxotremorine increased TH activity in A1, A2, and LC comparably in magnitude and time course to reserpine. However immunotitration demonstrated the increase was due to activation. We conclude that the noradrenergic neurons in the medulla of the A1 and A2 groups share comparable biochemical properties with respect to regulation of TH as do those of the LC. Such control may be of importance in the control of blood pressure.

(Supported by NIH grants HL18974, MH24285, and a NIH Postdoctoral Fellowship F05 TW 02382-01).

817 PROLONGED DEPLETION OF BRAIN NOREPINEPHRINE AFTER HYPOTHERMIC STRESS: RATE OF REPLETION VARIES WITH AGE AND BRAIN REGION. Sue Ritter* and Nancy L. Pelzer* (SPON: R. C. Ritter). Coll. Vet. Med., Wash. State Univ., Pullman, WA 99164.

Regional brain catecholamine concentrations were measured at 8 time intervals after hypothermic stress in 3 month and 7 month old male Sprague-Dawley rats. Hypothermic stress was produced by restraining rats in wire mesh cones and partially immersing them in 18°C water for 6 hrs. in order to lower body temperature. In 7 month old rats telencephalic norepinephrine (NE) concentrations were reduced to 50% of control and hypothalamic NE concentrations were reduced to 64% of control immediately after removal from the cold. Telencephalic dopamine (DA) was elevated by 25% at this time. Rate of recovery of NE to control values was more rapid in the telencephalon than in the hypothalamus. Telencephalic levels of NE were 88% of control by 6 hrs. and had returned to 100% of control by 24 hrs. Hypothalamic NE was only 67% of normal by 24 hrs. after stress and even at 48 hrs. post stress was only 88% of control. In the 3 month old rats NE levels returned to control values more rapidly than in the 7 month old rats. Both telencephalic and hypothalamic NE levels were normal by 15 hrs. post stress. However, the rate of repletion was still consistently slower in the hypothalamus than in the telencephalon.

The prolonged depletion of NE in the 7 month old rats after this severe stress suggests that brain NE synthesis may be suppressed during the post stress period. In order to begin testing this possibility deamination of brain catecholamines was blocked by administration of pargyline (100 mg/kg) immediately after removal from the cold. Pargyline treated animals displayed hypothalamic DA concentrations that were 221% of control and cortical DA concentrations that were 166% of control by 2 hrs. after pargyline injection. However, pargyline did not cause any elevation of hypothalamic or cortical NE above the levels of nonpargyline treated stressed rats. Failure of pargyline to elevate brain NE levels after stress suggests that although DA synthesis continues during the post stress period NE synthesis during that time is impaired.

In summary, three conclusions may be drawn from these experiments: (1) the rate of NE repletion after stress induced depletion appears to be inversely related to age or to an age related variable; (2) repletion of hypothalamic NE occurs more slowly than repletion of telencephalic NE; and (3) the prolonged time course of NE depletion after severe hypothermic stress appears to be due to impaired synthesis of NE from precursor DA. Ongoing work is investigating the involvement of dopamine-beta-hydroxylase in the specific suppression of NE synthesis after stress exposure.

818 CHANGES IN POSTERIOR HYPOTHALAMIC SELF-STIMULATION FOLLOWING EXPERIMENTAL CEREBRAL INFARCTION IN RATS. Robert G. Robinson and Floyd E. Bloom. Dept. of Psychiat., Johns Hopkins Sch. Med, Baltimore, M.D. 21205 and Salk Institute, San Diego, CA 92112.

Recent studies have demonstrated that cerebral infarction leads to anatomical and biochemical changes in catecholamine neurons in areas of the brain which are uninjured by local ischemia. In an effort to evaluate the behavioral significance of these post-stroke changes in catecholamines, intracranial self-stimulation (ICSS) became a phenomenon of particular interest because it is readily quantifiable and catecholamine neurons are thought to mediate the behavior. Bipolar stimulating electrodes were placed bilaterially in the posterior hypothalamus of rats. After the animals were shaped to self-stimulate at both electrode sites, the right middle cerebral artery was ligated. During the 25 day postoperative period, the rate of ICSS at specified current values was compared with preoperative values. At 2 days after operation, there was a 33% decrease in the maximum frequency of ipsilateral ICSS. However, by 8 days after experimental stroke, there was a 16% increase in the maximal rate of ICSS above the preoperative level. The maximal rate of ICSS then returned to the preoperative level by 20 days after surgery. The minimum current necessary to elicit the maximal rate of response also changed in a biphasic manner, that is, the minimum required current was greater than pre operative control levels until 8 days after operation but then dropped below control level until 20 days postoperative. In other words, until 8 days postoperative, the ipsilateral electrode was less rewarding than it was preoperatively, even though the animal was receiving more current, while from 8 to 20 days after cerebral infarction the same electrode was more rewarding than preoperatively even though the animal was receiving less current. There were no changes in the current or rate of response in the contralateral electrode. This change in the rewarding character of the ICSS was also reflected in electrode preference when the animals were allowed to stimulate each electrode ad libitum. Preoperatively they stimulated both sides with equal frequency and in an alternating manner, that is, stimulating each side 10-20 times before switching over to the other lever. The ratio of right to left lever presses was 1.0 ± 0.03 SEM preoperatively. This ratio fell to 0.5 by 2 days postoperative and then rose to 1.4 by 8 days postoperative. These results demonstrate that stroke leads to important behavioral changes and suggest that these behavioral changes may be the result of neurophysiological changes in post-stroke catecholamine neurons.

819 SYNAPTIC SPECIFICITY DURING THE DEVELOPMENT OF THE AVIAN SYMPATHETIC PREGANGLIONIC NUCLEUS. Leonard L. Ross., Arnold J. Smolen and Leo Cosio*. Dept. Anat., Med. Coll. Penna., Phila., Pa.19129

Removal of a portion of the afferent input of a population of neurons results in a compensatory synaptogenesis of the remaining input. This has been established for a number of systems both central and peripheral, in the adult and during the course of development. To analyze the principles governing deprivation-induced CNS synaptogenesis during development, the embryonic chick spinal sympathetic nucleus, the nucleus of Terni (NT), together with its bulbospinal monoaminergic innervation was chosen as an experimental system.

We have previously shown that the first synapses are evident in the N.T. at 10 days in ovo (d.i.o.). Synaptogenesis proceeds rapidly and the adult synaptic pattern is established by 10 d.i.o. The developmental sequence of monoaminergic uptake in the cord parallels that of synapse formation. Further, the monoaminergic input to the N.T. is exclusively bulbar in origin, there being no spinal aminergic neurons. Thus, cervical spinal cord transection before 10 d.i.o. will (1) prevent the N.T. neurons from receiving a portion of its input at a presynaptogenic stage and (2) test the ability of a non-aminergic input to replace an aminergic input.

In the present study cervical spinal cords were transected at 8 d.i.o. At 20 d.i.o. the embryos were perfused and their mid-thoracic spinal cords studied by ultrastructural quantitative stereology. In control animals at 20 d.i.o. 30% of the neuronal surface synapse coverage is by axodendritic synapses containing dense-cored vesicles which have been shown to be monoaminergic. In the transected animals, total synaptic coverage of the N.T. neurons was reduced by 40%. Most of the loss (70%) was in coverage by those axodendritic synapses containing dense-cored vesicles. There was no reduction in the number of neurons or in the total neuronal surface area (i.e., neuron size). Nor was there any increase in the coverage by synapses containing clear vesicles (non-monoaminergic).

Therefore, (1) a 40% reduction in the input (mainly monoaminergic) to the presynaptogenic N.T. neurons does not appear to affect their structural development. However, a functional defect in rat postsynaptogenic preganglionic neurons has been shown following spinal transection (Hamill, Bloom and Black, '76). And (2) propriospinal, non-monoaminergic axons are not induced to expand their synapse formation as a result of deprivation of the bulbospinal monoaminergic input.

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820 CELL DIVISION IN THE DEVELOPMENT OF ADRENERGIC NEURONS. Rothman,T.P., Gershon,M.D., and Holtzer,H.* Departments of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, PA 19174, and College of Physicians & Surgeons of Columbia University, New York, NY 10032.

Mature neurons are non-dividing cells. However, the temporal relationship between the acquisition of a given neuron's definitive characteristics and loss of the cell's ability to divide during development has not been worked out in many systems. The present study was undertaken to analyze this relationship in developing chick sympathetic ganglion cells. Definitive characteristics of these cells include their catecholamine (CA) content and membrane uptake mechanism for CA. CA can be detected by formaldehyde-induced fluorescence (FIF) in primary ganglion cells of the developing chick by 3 1/2 day's of gestation. After incubation with ^{3}H-norepinephrine (^{3}H-NE) all of these cells identified and examined by radioautography were labeled. Uptake of ^{3}H-NE is inhibited by desmethylimipramine. As the primary ganglion cells migrate to form paravertebral and prevertebral sympathetic ganglia and the adrenal medulla they continue to contain CA. Chick embryos between 1.5 and 15 day's gestation were injected with ^{3}H-thymidine (10-50μc) and, after varying survival times, processed for simultaneous demonstration of CA by FIF and ^{3}H-DNA by radioautography. Tissues were embedded in Epon and cut at 2μm so that overlapping cells would not be present in the sections. In some experiments ^{3}H-thymidine was given continuously for varying periods to determine when CA-containing cells withdraw from the cell cycle. In other experiments embryos were processed 2-4 hours after ^{3}H-thymidine-injection so that labeled cells would be fixed while still in S or G2 to determine if cells which already contain CA are able to divide. The first CA cells to leave the cell cycle did so between days 2 and 3. The last CA cells left the cycle between days 13 and 15. Some neurons which already contained CA replicated their DNA. This conclusion was supported by the electron microscopic observation that neurons (neuroblasts) containing perikaryal dense cored vesicles typical of CA-containing cells were labeled by ^{3}H-thymidine and were found in mitosis. Therefore, the correlation between acquisition of transmitter content and uptake mechanisms and withdrawal from the cell cycle is not exact. Either proliferative divisions follow acquisition of these definitive characteristics or these characteristics may be present in cells which still belong to an immature precursor population.

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821 PERIPHERAL AND CENTRAL CATECHOLAMINES IN SPONTANEOUS HYPERTENSION. Juan M. Saavedra, Horst Grobecker*, Virginia Weise* and Julius Axelrod. NIH, Bethesda, Md. 20014

The activity of the catecholamine synthesizing enzymes has been studied in specific areas of the brain and in the adrenal glands during the development of the spontaneous hypertension in the rat.

The activity of the epinephrine-forming enzyme, phenylethanolamine-N-methyltransferase (PNMT) was increased in the A1 and A2 areas of the brain stem in young (4 week-old) but not in adult (14 week-old) SH rats.

Decreased norepinephrine (NE) levels were found in a few specific nuclei of the anterior-hypothalamic-preoptic area and in the posterior hypothalamus. The decreased activity of dopamine-β-hydroxylase (DBH) in the same areas indicated that the synthesis of the neurotransmitter is decreased in these regions and a possible absence of noradrenergic neurons. The decrease in the formation of NE in specific brain areas can result in a reduced activation of central alpha receptors in the hypothalamus which may be related to the onset of hypertension. This phenomena could also explain the central mechanism of action of alpha stimulant antihypertensive drugs like clonidine and alpha-methyl-dopa. The activities of catecholamine biosynthetic enzymes (tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanol-N-methyltransferase) in the adrenal medulla were inhibited early in the development of the SH rats. Adult animals, however, showed enhanced tyrosine hydroxylase activity. This indicates a possible relationship between central and peripheral catecholamine metabolism in the development of hypertension.

822 SELF-STIMULATION AT LOW PULSING RATES IN RATS: SUPPORT FOR CATECHOLAMINE VIEW. E. Schmidt* (SPON: I. Perline) Arizona State Univ., Psych. Dept., Tempe, AZ 85281.

Acquisition of self-stimulation at MFB sites was tested with very short bursts of 60 Hz sine waves (40 msec, 2.5 cycles) delivered at a rate of two short bursts per second. A series of five bursts was delivered for each lever press in an automated shaping procedure. Only two of fourteen rats which proved to be self-stimulators reached a rate of 400 responses/hr by the third daily session. However, most rats began responding over the next nine sessions when (1) the current was increased from 100 to 120 μA, (2) the pulsing rate was increased from 2 to 5 bursts/sec, or (3) the length of each burst was increased from 40 to 180 msec. When retested at the original parameters, seven of the fourteen rats averaged 400 responses/hr over three sessions. Next well trained rats turned a small runged wheel at various turning force requirements (40, 150 & 180 g) to receive 60 Hz, 100 μA stimulation trains on an intermittent schedule. Work output was nearly identical at different turning forces, providing an effort based measure of reward. Work output increased as a function of the duration of each stimulation train (0.3, 0.9 & 2.7 sec). When these trains were broken into a series of short bursts, work output for several rats dropped only slightly to moderately over daily 15 min sessions.

The findings support the view of brain stimulation reward as resulting from catecholamine (CA) release, showing that the reward effect can be obtained at pulsing rates near or below the normal 5-10 spikes/sec firing rates of the CA systems in the brain. Clearly, higher stimulation frequencies or longer duration can potentiate the CA release effect. However, the marginal acquisition and maintenance of self-stimulation at 2 bursts/sec implies that the post-synaptic effect may endure as long as 500 msec. Models of CA inhibition of post-synaptic neurons through the mobilization of an amine-specific adenylate cyclase - cyclic AMP mechanism are supported by these behavioral findings.

823 APOMORPHINE REVERSES THE IPSILATERAL DEPRESSION OF CAUDATOPUTAMEN GLUCOSE CONSUMPTION OBSERVED AFTER UNILATERAL ELECTROLYTIC LESIONS OF THE RAT LATERAL HYPOTHALAMUS. William J. Schwartz. Lab. Neurophysiol., NIMH, Bethesda, Maryland 20014

Experiments using the [^{14}C] 2-deoxy-D-glucose (2-DG) technique for measuring regional brain glucose consumption (*Science* 187: 850) have shown that unilateral electrolytic lesions of the rat lateral hypothalamus (LH) result in decreased forebrain glucose consumption in structures ipsilateral and rostral to the lesion (*Nature* 261: 155). Regions affected are wide-ranging and include, among others, ipsilateral neocortex, caudatoputamen (CP), globus pallidus, and thalamus. This right-left metabolic asymmetry is present at three days and persists for at least fourteen days after the lesion. Moreover, the asymmetry of glucose consumption can be partially reproduced in some of these forebrain structures, *most significantly in CP*, by 6-hydroxydopamine (6-OHDA) injections (4 µg/2 µl) into the substantia nigra (SN).

These data suggest that after unilateral electrolytic LH lesions the resulting right-left asymmetry of at least one structure, the CP, might be due in part to destruction of the ascending dopaminergic nigro-striatal pathway at the level of the LH. As one test of this hypothesis, apomorphine, a dopamine receptor agonist, was administered to LH-lesioned animals to determine whether pharmacological stimulation of denervated striatal dopaminergic receptors could correct the CP metabolic deficit.

Sprague-Dawley albino rats underwent unilateral electrolytic lesions of LH; 14 d. later they were given apomorphine HCl (1 mg/kg) intravenously, followed by an intravenous pulse of 2-DG for the determination of the pattern of regional brain glucose consumption. In these apomorphine-treated lesioned rats, glucose consumption remained ipsilaterally depressed in neocortex, globus pallidus, and thalamus, but the right-left asymmetry of CP glucose consumption had disappeared (and had even reversed in anterior CP). Interestingly, when apomorphine was given to rats 3 d. after LH lesion, there was no effect on the metabolic asymmetry of any of the forebrain structures, including that of the CP.

Therefore, the observed asymmetry of glucose consumption in rat CP after unilateral electrolytic LH lesions can be (1) partially reproduced by 6-OHDA injections in SN, and (2) alleviated by intravenous apomorphine. The observation that a 1 mg/kg dose of apomorphine restores CP glucose consumption 14 d. after LH lesion but is ineffective 3 d. after lesion may possibly be explained by a leftward shift in apomorphine's dose-response curve as a result of the gradual onset of "denervation supersensitivity" of the post-synaptic dopaminergic CP receptors.

824 IN VIVO UPTAKE AND RETROGRADE TRANSPORT OF ANTIBODY TO DOPAMINE-ß-HYDROXYLASE BY CENTRAL NERVOUS SYSTEM NORADRENERGIC NEURONS. Michael A. Silver* and David M. Jacobowitz. Lab. Clin. Sci., NIMH, Bethesda, MD 20014.

Previous reports from this laboratory (Brain Res. 91: 165, 1975; Brain Res. 104: 390, 1976) demonstrated that an intravenous administration of specific antisera to dopamine-ß-hydroxylase (ADßH) resulted in the uptake of this immunoglobulin by sympathetic nerve fibers; intraocular injections suggested that a retrograde flow of dopamine-ß-hydroxylase (DßH) was taking place. The purpose of this study was to determine whether a similar, specific transport system could be detected in DßH-containing noradrenergic neurons of the rat CNS. ADßH or pre-immune serum were stereotaxically injected into either the lateral ventricle (LV, 20 µl) or the medial forebrain bundle - dorsal bundle (MFB-DB, A5150, 2 µl). Animals were sacrificed at 3 hrs to 14 days post injection. Cryostat sections were fixed in chloroform:methanol (2:1) and stained with fluoroscein conjugated IgG. An intense bilateral granular fluorescence was observed 1 day after LV administration of ADßH within processes and cell bodies of the locus coeruleus. This technique also permitted the identification of the ascending dorsal noradrenergic bundle arising from the locus coeruleus in agreement with previous reports. At 3- and 6-hrs, the first detectable fluorescence was observed in fibers of the dorsal bundle. Some cell bodies appeared to fluoresce 10-hrs post-injection. An intense fluorescence in the locus coeruleus was maintained for 4 days but was absent by day 8. Bilateral transection of the dorsal bundle in the rostral mesencephalon at the time of injection effectively blocked the retrograde transport of fluorescent material to the locus coeruleus. Unilateral injection of ADßH into the MFB-DB resulted in bilateral labelling of both the dorsal and ventral noradrenergic bundles, as well as the A_1, A_2, A_4, A_5, locus coeruleus and subcoeruleus cell groups. Control pre-immune serum injections did not result in the fluorescence labelling of any cell groups or fibers. The specificity of this technique is supported both by results in agreement with previous catecholamine histofluorescence and immunocytochemical studies and by a lack of fluorescence in other cell body regions. The significance of a retrograde flow of DßH is unknown. In conclusion, this technique combines retrograde transport of a marker protein, with the sensitivity and specificity of immunocytochemical procedures to provide a new tool for the neuroanatomical study of neurotransmitter systems.

825 MEDIAL FOREBRAIN RESPONSE TO BRAIN STIMULATION REWARD IN RATS. Harry M. Sinnamon, Laboratory of Neuropsychology, Wesleyan University, Middletown, Ct. 06457.

Similar to barpressing rates, the response pattern of most single units in the medial forebrain (<2.5mm from midline) to activation of intracranial self-stimularion (ICSS) sites depends on the pulse frequency of stimulation. A frequency of 100 Hz is far more effective than either 50 or 25 Hz. For recording, rats with chronic stimulation electrodes in verified ICSS sites in the lateral hypothalamus and ventral tegmental area were anesthetized and maintained with minimal levels (0.5%) of halothane for acute recording. Single trains of 50 pulses at 100 Hz (15 sec between trains) changed the activity pattern of the vast majority of 200 units in the anteromedial cortex, anterodorsal cortex, striatum, septum, accumbens and rostral MFB. Most areas had units that responded during the train with either excitation or inhibition. Biphasic patterns (inhibition-facilitation and facilitation-inhibition), and increases in activity lasting several sec after a train were responses especially common in the cortex and striatum. The striatum was also particularly rich in units responding to single pulses with short latency (<20msec) excitation. Biphasic responses and longlasting increases in activity follow time courses similar to the "drive-like" aftereffects that are prominent in ICSS reward.

826 A NEW GROUP OF CATECHOLAMINE-CONTAINING CELLS (A-15?) IN THE RAT DIENCEPHALON. John R. Sladek, Jr. and Thomas H. McNeill. University of Rochester School of Medicine, Rochester, New York 14642.

Catecholamine-containing neurons (A-1 through A-14) have been localized to the mammalian brain stem and hypothalamus. The hypothalamic groups occupy arcuate, periventricular, and other regions. Non-mammalian vertebrates possess an additional group of catecholamine-containing cells located subependymally within the diencephalon. Such cells have not been reported, however, in mammals. The present investigation represents the search for catecholamine-containing subependymal cells within a member of the mammalian order Rodentia.

Formaldehyde- and glyoxylic acid-induced histofluorescence was examined in the avian (duck, pheasant) and mammalian (rat) brain. Catecholamine- and serotonin-containing cells are known to exist subependymally in the avian brain, principally within the paraventricular organ. A dual population of intense blue and yellow cells was seen lining the wall of the avian third ventricle. Microspectrofluorometric analysis characterized the blue fluorophor as a catecholamine, and the yellow fluorophor as the indoleamine, serotonin. Similar cells were not seen routinely in the rat brain following formaldehyde histochemistry. However, glyoxylic acid histochemistry revealed a previously undescribed population of catecholamine-containing subependymal cells which lined the entire third ventricle of the rat diencephalon. The cells were 10µm in diameter and possessed two fluorescent processes, one extending toward and the other away from the third ventricle. They yielded a blue fluorescence which was characteristic of a catecholamine as analyzed spectrally. Additional animals were pretreated pharmacologically with a combination of reserpine, L-dopa, and a peripheral dopadecarboxylase inhibitor (MK486). This treatment resulted in the persistence of the catecholamine fluorescence of dopaminergic (e.g., median eminence-contact zone, arcuate nucleus), but not noradrenergic (e.g., supraoptic nucleus) areas of the hypothalamus. Following this treatment, the subependymal population of catecholamine cells also retained their fluorescence, indicating the probability that they are dopaminergic. The location of these cells along the ventricular wall is reminiscent of monoamine-containing subependymal cells of non-mammalian vertebrates and may represent a species homologie in the mammal. These cells are not to be confused with arcuate (A-12) and periventricular (A-14) dopamine neurons. Ultrastructural identification is needed to define the cells as neuronal, ependymal, or otherwise.

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827 LOCALIZATION OF AVIAN BULBOSPINAL MONOAMINERGIC NEURONS BY FLUORESCENCE HISTOCHEMISTRY AND RETROGRADE TRANSPORT OF HRP. Arnold J. Smolen, Ellyn J. Glazer and Leonard L. Ross. Dept. of Anat., Med. Coll. Pa., Philadelphia.,Pa. 19129.

We have recently studied the synaptic arrangement within the preganglionic sympathetic nucleus of the chicken (Nucleus of Terni, NT), and have described the existence of boutons in the neuropil which represent the bulbospinal noradrenergic and serotonergic pathways. In the present study the cell bodies of origin of these bulbospinal projections are identified.

Catecholaminergic (CA) and serotonergic (5-HT) cell bodies were identified in the brainstem by fluorescence histochemistry. Other animals received a thoracic spinal cord injection of 0.4 µl of a 33% solution of HRP (Sigma Type VI). The animals were sacrificed after 24 hours. Serial sections through the brainstem were cut at 50 µm on a Vibratome. The sections were then reacted with sodium nitroferricyanide, benzidine dihydrochloride and hydrogen peroxide according to the method recently described by Mesulam (J. Histochem. Cytochem. 24, 1976). This results in a blue granular reaction product within labeled neurons, which contrasts with the neutral red counterstain, and makes possible unambiguous localization using bright-field microscopy.

Localization of fluorescing perikarya in the brainstem was found to correspond with that already described for the chicken by Ikeda and Gotoh (Jap. J. Pharmacol. 21, 1971). One group of HRP labeled neurons was found in the caudal and ventral portion of the medullary raphe which corresponds to the 5-HT group B2. Another group of neurons containing HRP was found in the ventrolateral reticular formation at the level of the closed medulla corresponding to the CA group A1. A third group of HRP labeled cells was found in proximity to the dorsal motor nucleus of the vagus and nucleus solitarius, also at the level of the closed medulla. This group corresponds to the CA cell group A2. Finally, there was a small group of labeled neurons in the ventrolateral reticular formation at the level of the abducens nucleus corresponding to the CA group A4. Other brainstem areas,in addition to these monoaminergic groups, were also labeled with HRP following thoracic spinal cord injection. These areas include the inferior olivary nucleus, the reticular formation, the vestibular complex, and the red nucleus.

It may be concluded that the noradrenergic innervation to the avian thoracic spinal cord arises from cell groups A1 and A4 in the ventrolateral medullary reticular formation, and A1 more dorsally in the region of the vagal nuclei. The serotonergic projection arises from the caudal portion of the ventral raphe, group B2.

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828 CONTRIBUTION OF LOCUS COERULEUS-NORADRENERGIC SYSTEM TO CARDIO-ACCELERATION IN NONHUMAN PRIMATES. Daniel R. Snyder, Y.H. Huang, and D.E. Redmond, Jr., Neurobehavioral Lab., Sect. Comp. Med., and Dept. Psychiatry, Yale Univ., New Haven, Ct. 06510.

Following bilateral lesions of the nucleus locus coeruleus (LC), chair-restrained *M. arctoides* failed to show the cardio-accelerator response of normal and sham operated monkeys to threatening stimuli. In addition to absence of increased heart rate, lesioned monkeys also failed to show generalized excitement or escape behaviors when receiving or anticipating the receipt of inescapable shock. Standardized, threatening stimuli used to test cardiovascular responsivity included directed threat from a human, blood pressure cuff inflation, and punishment from a hand-held electrical shocking device. The fact that bilateral LC lesions do not affect resting pulse rate suggests that the locus coeruleus affects cardiovascular function primarily in response to environmental events such as threats or stress. This is consistent with the reduced emotional behavioral response to threats after LC lesions (Huang, et al, NEUROSCI. ABS. 2: 489, 1976).

Electrical stimulation of the LC causes complex cardiovascular responses (Przuntek and Philipu, ARCH. GES.PATH.PHARMAK. 276: 119, 1973; Ward and Gunn, BR. RES. 107:401, 1976) which contrast with the effects of precise bilateral LC lesions. These data are consistent with the hypothesis that the LC mediates specific behavioral and cardiovascular responses which are components of an alarm or fear reaction to threatening stimuli.

829 MATERNAL BEHAVIOR IN THE FEMALE RAT: CONTROL BY THE NORADRENERGIC FIBER SYSTEM. Marianne K. Steele, David L. Rowland and Howard Moltz*. Dept. of behavioral Sciences, University of Chicago, Chicago, Ill. and Millikin University, Decatur, Ill.

The noradrenergic fiber systems in the brain were manipulated in primiparous female rats, 6-10 days post coitum. Lesions of the far-lateral hypothalamus, resulting in depletion of hypothalamic NE, produced deficits in maternal behavior when observed over a two week testing period. Brainstem lesions of the dorsal NE bundle resulted in a loss of hippocampal NE which was similarly associated with deficits in pup care. In contrast, 6-OHDA infusion into the ventral tegmentum produced profound depletions of NE in cortex, hippocampus and hypothalamus with no observable detrimental effects upon maternal behavior. However, direct manipulation of the fiber connection (fornix) between the hippocampus and hypothalamus resulted in deficits in maternal behavior and a loss of NE from the hippocampus.

These data therefore suggest that a balanced noradrenergic relationship between the hippocampus and the hypothalamus is critical for the onset of maternal behavior. Depletion in either one or the other of these brain areas disrupts the initiation of pup care in the primiparous female rat.

830 CONTRIBUTION OF HISTAMINERGIC AND ALPHA ADRENERGIC RECEPTORS TO ENDOGENOUS CAMP PRODUCTION IN RAT CEREBRAL CORTEX: Anita A. Suran, Dept. Pharmacol., Coll. Med., Howard Univ., Washington, D. C. 20059.

CAMP production was measured in cortical slices prelabelled with ^{14}C-Adenine [J. Neurochem 16, 1609 (1969); the procedure was modified by incubating in air, using 0.05 M Tris buffer at pH 7.4 and 0.2% glucose]. Histamine-stimulated-CAMP accumulation (H), optimal at 1 mM, ranged from 150-200% of basal values (B). Using methoxamine as an alpha agonist, methoxamine-stimulated-CAMP production (M) averaged about 110% B, and was optimal at 0.1 mM. The H1 and H2 antagonists pyribenzamine (Py) and cimetidine (Ci) at concentrations in the range 0.01-10 uM depressed H to 62% B and 83% B respectively; similiarly, M was reduced by 10 uM Py to 80% B and by 10 uM Ci to 84% B. In the absence of agonist, B was depressed to 83% by Py and to 83% by Ci, and using alpha antagonists values were reduced to 81% by phenoxybenzamine (Phb) and to 65% B by phentolamine (Pht); B was reduced by Phb to 95% and by Pht to 90%. 0.1 mM clonidine (Cl), an alpha agonist, stimulated CAMP to 110% B, and significantly potentiated the inhibitory effects of antagonists to both histamine and methoxamine, reducing B to 48% by Ci, to 60% by Py, to 75% by Phb. Both histaminergic and alpha adrenergic stimulated CAMP accumulations, as well as B, were inhibited by H1, H2, and alpha antagonists, and the depression in CAMP accumulation caused by agonist plus antagonist was equal to or greater than with antagonist alone. To investigate the nature of the enhanced antagonisms and to differentiate histamine and methoxamine specificities of the cortical system, B was partially depressed by a mixture of Py and Ci, each 1 mM, then increasing amounts of histamine or methoxamine were added. It was anticipated that the antagonism would be competitively reversed; quite the opposite was observed. In a dose response, histamine, in the range 1-20 mM, increasingly diminished CAMP levels to 20% B at 10 and 20 mM; methoxamine in the range 0.1-5 mM produced a decrease to 20% B. In the presence of 10 uM Pht, histamine (1-5 mM) caused a maximal decrease to 39% of B, and methoxamine (0.1-5 mM) decreased CAMP to 64% B. In the antagonist-free system, 10 mM histamine- or 5 mM methoxamine- stimulated CAMP accumulations were only slightly less than optimal so that high agonist concentrations per se are not inhibitory; therefore, it appears that a true potentiation of the antagonist activity was observed. The dose responsive potentiated antagonism suggests involvement of altered or "new" receptors for histamine or methoxamine, which become available only in the presence of inhibitors which occupy the "normal"agonist sites mediating CAMP accumulation. In this system an interrelationship among histaminergic and α-adrenergic receptors has been demonstrated.

831 VALINE-INDUCED ALTERATIONS OF ENHANCED 5 HYDROXYINDOLE SYNTHESIS THAT OCCURS FOLLOWING PRECURSOR LOADING OR SYNAPTIC RECEPTOR BLOCKADE. R.F. Thomas, J.J. Poulakos*, A. Siegel and J.H. Jacoby, Depts. of Pharmacology and Anatomy, CMDNJ, New Jersey Medical School, Newark, N.J. 07103.

Plasma neutral amino acids that compete with tryptophan for uptake into brain prevent the subsequent rise of brain tryptophan and 5-hydroxyindoles (serotonin + 5-hydroxyindole-acetic acid) that might otherwise occur (Fernstrom and Wurtman, Scient. Am. 230: 84, 1974). We have made use of the administration of a neutral amino acid i.e., valine, concomitant with either precursor loading with tryptophan or serotonin receptor blockade with methiothepin (which consequently leads to a compensatory increase of serotonin synthesis) to determine the ability of such a pharmacologic manipulation to compete with tryptophan for uptake into brain and to impair the subsequent acceleration of 5-hydroxyindole synthesis. Fasted male Sprague-Dawley rats (135-150g) were injected with saline or methiothepin (20 mg/kg) followed 15 min. later with tryptophan (50 mg/kg) and varying doses of valine, and killed 60 min. later. Increasing concentrations of valine decreased brain accumulation of tryptophan in a dose-related manner and decreased the subsequent rise of brain 5-hydroxyindoles in a non-dose related manner. When tryptophan was administered to methiothepin pretreated animals, the subsequent elevation of brain tryptophan was much higher than observed following a similar dose of tryptophan without methiothepin. Valine (600 mg/kg) when given together with tryptophan, prevented the methiothepin-induced enhancement of tryptophan uptake into brain, yet total brain 5-hydroxyindoles levels were still higher than similarly treated animals not receiving methiothepin. However, the reduction of total brain 5-hydroxyindoles induced by valine given to either tryptophan loaded rats with or without methiothepin pretreatment was the same. These results suggest that competition with tryptophan for uptake into brain can effectively diminish 5-hydroxyindoles production even during states of enhanced synthesis, and that the effects of drugs acting upon brain serotoninergic systems may be altered by changes in peripheral amino acid availability.

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832 EFFECT OF P-CHLOROAMPHETAMINE ON CATECHOLAMINE CONCENTRATION OF DISCRETE BRAIN AREAS. Y. Tizabi*, V. John Massari* and D. M. Jacobowitz (SPON: R. M. Kostrzewa). Dept. Pharmacology, Howard Univ., Washington, D.C. 20059; and Lab. Clin. Sci., NIMH, Bethesda, MD 20014.

P-Chloroamphetamine (PCA) has been shown to have a long term and irreversible neurotoxic effect on serotonergic systems of the brain. A single dose of PCA in rats reduces the brain serotonin content for at least a period of 4 months. Its effects are believed to be mainly restricted to serotonergic nerve terminals. Morphological and biochemical data also suggest that the drug is toxic to neurons of the B-9 cell group. PCA has also been shown to have a short term effect on brain catecholamine (CA) turnover. Recently, however, Hattori et al. (Neurochem. Res. 1: 451, 1976) have reported that administration of 10-15 mg/kg i.p. PCA causes the ultrastructural appearance of two types of terminal degeneration in the striatum of rats. They postulate that this is due to the destruction of both dopaminergic and serotonergic axon terminals. We have studied the effect of PCA on CA content of the striatum and other discrete areas of the brain. Male Sprague-Dawley rats were injected with 10 mg/kg i.p. PCA or saline and were sacrificed 3 or 9 days after the injection. Brains were quickly removed, frozen, and cut into 300 μm thick slices. Discrete areas (arcuate nucleus, striatum, A-10 cell body area, substantia nigra, central grey CA area, A-5 and locus coeruleus) were punched out for norepinephrine (NE) and dopamine (DA) measurements. The arcuate nucleus of the 3 day treated rats showed a significant decrease (48%) in DA content when compared with its control. This difference was absent in the 9 day treated rats. There was no significant difference in either NE or DA content of any other area at either time interval under study. In conclusion, these results do not support the hypothesis that PCA is also toxic to dopaminergic neurons.

833 CORTICOSTERONE MODULATION OF THE RESPONSES OF NIGRONEOSTRIATAL AND TUBEROINFUNDIBULAR DOPAMINE NEURONS TO RECEPTOR BLOCKADE. Glen R. Van Loon* and Chul Kim* (SPON: J.W. Scott). Department of Medicine, University of Toronto, Toronto, Canada M5S 1A8.

Previous studies have failed to demonstrate clearly an interaction between corticosterone and central dopaminergic neurons. Recent studies from our laboratory have suggested that alterations in plasma ACTH and corticosterone are associated with changes in brain dopamine (DA) metabolism. Hypophysectomy in rats results in increased concentration of homovanillic acid (HVA) in ventral hypothalamus (VH); this increase is entirely prevented by ACTH administration. These findings were not duplicated in striatum. In the present studies, we have examined the effect of corticosterone (B) administration on the HVA response in VH and the dihydroxyphenylacetic acid (DOPAC) and HVA responses in striatum to the DA receptor blocking agent, haloperidol (HPD). Although several previous studies have examined the effect of HPD on DOPAC and HVA in nigroneostriatal DA neurons, no similar studies have been performed on tuberoinfundibular (TI) DA neurons. HPD, 0.1 mg/kg, produced a maximal increase in striatal HVA when examined 2 hr later. HVA increased from 0.72 ± 0.03 to 1.32 ± 0.04 ug/g ($p<0.001$); increasing HPD further to 0.7 mg/kg failed to produce any further increase in striatal HVA. Similarly HPD, 0.1 mg/kg, produced a maximal increase in striatal DOPAC from 0.81 ± 0.02 to 2.01 ± 0.05 ug/g($p<0.001$). Administration of B, 20 mg/kg but not 7.5 or 2 mg/kg, inhibited both the HVA and DOPAC responses to HPD, 0.1 mg/kg. The dosage of HPD necessary to increase HVA maximally in the presence of B, 20 mg/kg, was increased to between 0.1 and 0.3 mg/kg. HPD, 0.1 mg/kg, produced a maximal increase in HVA in VH also, from 0.061 ± 0.005 to 0.086 ± 0.008 ($p<0.05$). B, 20 and 7.5 mg/kg but not 2 mg/kg, inhibited the HVA response to HPD, 0.1 mg/kg. The dosage of HPD necessary to increase HVA maximally in the presence of B, 20 mg/kg, was increased to between 0.3 and 0.7 mg/kg. Thus the dose of B necessary to inhibit the HVA response to HPD is lower in VH than in striatum, and the dose of HPD necessary to overcome the inhibitory effect of B, 20 mg/kg, is greater in VH than in striatum. Furthermore, B alone in absence of HPD decreased basal HVA concentration in VH (0.038 ± 0.003 ug/g;$p<0.05$), but not in striatum (0.69 ± 0.02 ug/g). Thus the TI DA neurons appear more sensitive than nigroneostriatal DA neurons to B. It seems likely that corticosterone produces this effect by binding to pre- or postsynaptic DA receptors, especially since the interactions between B, HPD and HVA appear dose-related. These data provide firm support for an interaction between corticosterone and brain DA metabolism, and raise the possibility that TI DA neurons play a role in the feedback regulation of ACTH secretion by corticosterone. (Supported by MRC MA-5183 and OMHF 534-75B)

834 EFFECT OF ADRENERGIC STIMULATION AND ESERINE ON THALAMOCORTICAL RECRUITMENT. William G. VanMeter, Dept. Vet. Anat., Pharmacol., and Physiol., Col. Vet. Med., ISU, Ames, IA 50011.

EEG activity was monitored and the effect of adrenergic stimulation on thalamocortical recruitment (T-C rct) was observed in restrained, conscious albino rabbits and encephale-isolé cats. Recruiting responses were analyzed by computer averaging techniques for comparison of treatment effects. MAOI by pargyline in amounts to 12.5 mg/kg i.v. was found inadequate to antagonize T-C rct and did not potentiate the effect of a subthreshold dose of Eserine (50.0 mcgm/kg i.v. as base) on the evoked responses but induced a desynchronization of the EEG. Approximately 50.0 mg/kg i.p. of L-DOPA was required to induce a slight attenuation of T-C rct and this effect was noted 60 minutes post injection. The effect of the subthreshold dose of Eserine was slightly enhanced and an EEG desynchronization was induced. Treatment with pargyline (12.5 mg/kg i.v.) and L-DOPA (20.0 mg/kg i.p.) was found to be the optimal combination of doses to enhance the antagonism of T-C rct by Eserine (50.0 mcgm/kg i.v.).

Amphetamine given in amounts to 20.0 mg/kg i.v. was found to induce an EEG desynchronization but failed to attenuate T-C rct. However, a marked potentiation of the subthreshold dose of Eserine was routinely observed.

Chlorpromazine (3.5 mg/kg i.v.) failed to antagonize the evoked responses while D-INPEA in doses of 4.0 mg/kg i.v. revealed a slight attenuation of T-C rct.

835 NOREPINEPHRINE IS THE MONOAMINE OF THE SIF CELLS OF THE GUINEA PIG SUPERIOR CERVICAL GANGLION: IMMUNOCYTOCHEMICAL DEMONSTRATION. James K. Wamsley*, Asa C. Black Jr., Jan Redick*, James R. West*, and Terence H. Williams. Dept. Anat., Univ. Iowa, Iowa City, Iowa 52242.

We have studied the morphology of the small, intensely fluorescent (SIF) cells of the guinea pig superior cervical ganglion (SCG) using the glyoxylic acid procedure of Chiba and Williams (1). Clusters of SIF cells were seen in close proximity to blood vessels. Varicose processes of varying length were seen emanating from some SIF cells; many of these passed directly to nearby blood vessels, while others ran toward the principal ganglionic neurons. The SIF cells localized an antibody to dopamine β-hydroxylase, using the fluorescein isothiocyanate method of Hartman (2). A second group of sections exhibited localization of dopamine β-hydroxylase to the SIF cells, using the peroxidase-antiperoxidase technique of Sternberger (3). None of the SIF cells exhibited localization of phenylethylamine-N-methyl transferase, demonstrating that epinephrine is not the transmitter for SIF cells in the guinea pig SCG. Building upon the observations of Libet (4), Greengard and his coworkers have developed a model of the role of cyclic AMP in neural transmission in the rabbit and cow SCG (5). This model involves a dopaminergic SIF cell/interneuron which liberates dopamine at its synapse with the principal ganglionic neuron, causing increased intracellular generation of cyclic AMP, and generation of a slow inhibitory postsynaptic potential (s-IPSP). The guinea pig SCG seems to differ in several respects from the rabbit, however. Its SIF cells contain norepinephrine rather than dopamine; moreover, the guinea pig SCG does not exhibit a s-IPSP (4). Incubation of guinea pig SCG with exogenous dopamine in vitro did not lead to elevation of cyclic AMP levels. However, incubation in vitro with isoproterenol led to marked increases in cyclic AMP levels in the guinea pig SCG, indicating the existence of a β-adrenergic receptor--adenylate cyclase complex in this species. We hypothesize that the presence of a β-adrenergic receptor--adenylate cyclase complex rather than a dopamine receptor--adenylate cyclase complex may be related to the presence of norepinephrine as a transmitter in this species. Elucidation of the mechanisms underlying SIF cell function in the guinea pig SCG may lead to better understanding of the role of SIF cells in ganglionic transmission. (1) Cell Tis. Res., 162:331-341. 2. J. Histochem. Cytochem., 21:312-332. 3. J. Histochem. Cytochem., 18:315-333. 4. Fed. Proc., 29:1945-1956. 5. Fed. Proc., 33:1059-1067. Supported by NS-11650 to T.H.W., and by a PMA Foundation Research Starter Grant and G.R.S. from the University of Iowa to A.C.B.

836 PHYSIOLOGICAL AND ANATOMICAL EVIDENCE FOR SEROTONERGIC AXON COLLATERALS IN THE RAT MIDBRAIN RAPHE. Rex Y. Wang and George K. Aghajanian, Depts. Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508

A number of previous studies have suggested the existence of serotonergic (5-hydroxytryptamine; 5HT) axon collaterals in the midbrain raphe nuclei. To test for collateral influences directly, antidromic responses were evoked in 5HT cells of the midbrain raphe by stimulation of the major ascending 5HT pathway in the midline of the ventromedial tegmentum (VMT). Experiments were done in cerveau isolé or chloral hydrate anesthetized rats. To rule out the possibility of simultaneous stimulation of afferent fibers, some experiments were performed on animals with various lesions including pre-VMT-transected (forebrain deafferented) rats. To determine whether VMT-induced effects are mediated through antidromic invasion of collaterals in the 5HT pathway, 5,7-dihydroxytryptamine (5,7-DHT; 10 μg in 5 μl) was injected directly into the VMT area to destroy selectively 5HT axons; 6-hydroxydopamine pretreated rats served as controls. Parallel degeneration studies were carried out at both the light (cupric-silver staining) and electron microscopic level 24-48 hrs after the injection of 5,7-DHT (200 μg) into the lateral ventricle. Rats were pretreated with a norepinephrine (NE) uptake blocker, desipramine (DMI; 25 mg/kg) to protect the NE system. In all 5HT cells studied (except those cells recorded from 5,7-DHT pretreated rats) there was a period of suppressed firing following stimulation of the VMT. The duration of the suppression was proportional to the stimulus intensity, indicating that this effect was due to synaptic summation rather than the refractory period of the cell membrane. The suppression was prevented when 5HT axons in the VMT were destroyed but not when other systems were lesioned. Iontophoresis of 5HT directly onto identified 5HT cells invariably produced inhibition. Both the 5HT inhibition and the post-stimulus inhibition were potentiated by iontophoretic application of a 5HT uptake blocker (chlorimipramine) but not by a NE uptake blocker (DMI) or a γ-aminobutyric acid receptor antagonist (picrotoxin). In addition to the physiological evidence for direct 5HT collateral inhibition, degenerating 5HT axon terminals were observed making specialized synaptic junctions onto disintegrating 5HT cell bodies and dendrites after intraventricular 5,7-DHT. In conclusion, the results indicate the existence of 5HT axon collaterals and that these exert an inhibitory influence upon 5HT neurons, presumably via "autoreceptors". This self-regulating system may have a role in maintaining the slow, regular firing of 5HT neurons; it may also underlie the fact that 5HT neurons are highly sensitive to drug and precursor induced changes in the availability of 5HT. (USPHS Grant MH-17871 and the State of Connecticut).

837 DECREASED LOCOMOTOR ACTIVITY AND ATTENUATION OF AMPHETAMINE-INDUCED HYPERACTIVITY WITH INTRACEREBROVENTRICULAR INFUSIONS OF SEROTONIN IN RAT. John D. Warbritton, III,* R. Malcolm Stewart and Ross J. Baldessarini. Dept. of Psychiatry, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114 and McLean Hospital, Belmont, MA 02178.

Serotonin (5-HT) is implicated as an inhibitory neurotransmitter in the central nervous system (CNS), with behaviorally depressant effects. Continuous (150 min) intracranial infusion of 5-HT into the right lateral cerebral ventricle of male rats through a chronically implanted cannula diminished motor activity recorded with a Stoelting electronic activity monitor. This effect was dependent on the dose of 5-HT infused, from 2 to 15 μg/min (at 1.5 μl/min); higher doses of 5-HT produced toxic side-effects, including generalized seizures and death. The approximate half-maximally effective dose (ED_{50}) of 5-HT was 5 μg/min. Saline infused at up to 10 μl/min had only small excitatory effects. Acute administration of d-amphetamine (2.5 mg/kg, i.p.) markedly increased locomotor activity without inducing stereotypy. This hyperactivity was attenuated by the intraventricular infusion of 5-HT (ED_{50}=7.5 μg/min). Parallel decreases in both spontaneous and amphetamine-stimulated activity following the direct infusion of 5-HT into the lateral ventricle supports the view that 5-HT functions as a behaviorally inhibitory neurotransmitter in the mammalian CNS.

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838 STUDIES ON THE ROLE OF THE NUCLEUS ACCUMBENS IN AMPHETAMINE INDUCED LOCOMOTION. David Wirtshafter, Karen E. Asin and Ernest W. Kent. Dept. Psych., Univ. Il. at Chicago Circle, Chicago, Il. 60680.

Recently a number of investigators have suggested that the hyperkinetic effects of amphetamine (AMPH) may be mediated through the release of dopamine in the nucleus accumbens (NAc). In support of this contention we have observed (Neurosci. Abst. II,#738) that injections of 6-hydroxydopamine (6-OHDA) into the olfactory tubercle at the ventral border of the NAc severely attenuate the locomotor response to amphetamine. In the studies reported in the current communication we have attempted to further clarify the functional role of the NAc by examining the AMPH response in rats with large electrolytic lesions of the NAc.

Relative to controls, animals in which 80-90% of the NAc was destroyed displayed a marked increase in spontaneous motility. In contrast to animals with 6-OHDA lesions, however, 2 mg/kg d-AMPH produced a clear increase in locomotion in these subjects. To test the possibility that this response was mediated through the activation of dopamine receptors in the remaining portions of the NAc, we examined the response of lesioned and control animals to 1 mg/kg of the direct dopamine receptor agonist apomorphine. Apomorphine produced an immediate increase in activity in control subjects but was almost without effect in NAc lesioned animals. This result was obtained even when subjects were allowed 4.5 hours to habituate to the photocell box in which activity was measured; at this time the activity baseline of lesioned and control animals was similar. The finding that direct dopamine receptor activation in NAc lesioned animals had little effect on locomotion suggested that the persistent AMPH response may have been mediated through the release of noradrenalin rather than dopamine. In support of this view we have found that AMPH hypermotility in NAc lesioned animals can be suppressed by phenoxybenzamine or diethyldithiocarbamate at doses which have little effect in intact animals.

It seems rather paradoxical that 6-OHDA lesions of the NAc abolish AMPH induced activity whereas electrolytic lesions do not. We would like to suggest that in the absence of a dopaminergic innervation the NAc may exert some sort of inhibitory influence on locomotion which may override the effects of increased noradrenalin release produced by AMPH. Electrolytic NAc lesions, by removing this inhibition, might allow noradrenalin release in other parts of the nervous system to influence activity.

839 ATTENUATION OF FOOD REWARD BY DOPAMINE RECEPTOR BLOCKADE IN RATS. Roy A. Wise. Center for Research on Drug Dependence, Dept. Psychology, Concordia Univ., Montreal, Canada H3G 1M8.

Lever-pressing for food was attenuated by pimozide (0.5-2.0 mg/kg) in hungry rats. Initial responding was normal and subsequent responding was only minimally attenuated on the first pimozide test in naive animals; thus drug-induced ataxia was not significant. Responding was progressively more attenuated in subsequent pimozide tests. Parallel effects were seen with conditions of non-reward. Initial responding was normal and subsequent responding was only minimally attenuated on the first day of non-rewarded testing in naive animals. Responding was progressively more attenuated as animals received experience in subsequent non-rewarded testing. The parallel effects of pimozide and non-reward experience suggested an equivalence of pimozide and non-reward conditions. This seemed confirmed by the additional finding that responding was minimal even on the first day of pimozide testing in animals that had prior experience with non-reward.

Normal responding under pimozide at the beginning of sessions and for the whole first session in naive animals rules out any pimozide-induced motor or performance difficulties. Thus pimozide appears to block the rewarding quality of food for hungry animals, just as it appears to block the rewarding qualities of brain stimulation and intravenous amphetamine and cocaine. These data suggest that a common dopaminergic substrate plays a critical and perhaps specialized role in mediation of reward. It is paradoxical that dopamine receptor blockers should block the impact of hedonic (rewarding) stimuli in animals, since they are used in man to alleviate the symptoms of schizophrenia, one of which is reported to be anhedonia.

840 ALTERNATE SECTION HISTOFLUORESCENCE/CYTOCHEMISTRY OF IDENTIFIED AMINERGIC NEURONS. Joe Wood and John R. Sladek Jr., Dept. of Neurobiology & Anatomy, Univ. of Tex. Med. Sch., Houston, Tex. 77025; Dept. of Anatomy, Univ. of Rochester, Rochester, N.Y. (SPON: T.F. Burks).

The correlation of fluorescence microscopic and electron microscopic identification of amine containing neurons is difficult due to methodological incompatibilities. Both paraformaldehyde and glyoxylic acid (GA) induced histofluorescence render tissues nonadaptable for specific cytochemical amine localization. Additionally, electron microscopic glutaraldehyde-dichromate (GDC) treatment for amines prevents any histofluorescence reaction. Therefore, a method which can utilize the same brain where one technique can be compared against the other on identified nuclear groups or single identified aminergic neurons is needed. Rat brains were perfused with cold Ringer's solution. Brain sections (30 μ) were made on a Vibratome @ 1-2°C for light microscopy (both histofluorescence and GDC) and at 300 microns for electron microscopy. 30μ sections were immersed in 2% GA for 2 min, air dried at 45°C for 15 minutes and baked for 5 minutes at 100°C with one gram of GA per coplin jar. Tissues for GDC were soaked 1 hr in 3% glutaraldehyde then transferred to the GDC mixture (pH 4.1). GDC tissues were also processed for electron microscopy. Brain areas examined were the arcuate nucleus (A-12), the median eminence, the median raphe and the substantia nigra (SN). Identifiable positively fluorescing neurons can also be identified in thick sections at the light microscopic level with the GDC technique. The same cell areas are positive when viewed with the electron microscope. In the median raphe, fibers containing sharp puncate varicosities were seen with fluorescence and similar fibers were seen in GDC sections. This technique provides the advantage of the enhancement of the histofluorescence for point to point evaluation of neuron relationship. Thus, even when histofluorescence itself does not have the capability of demonstrating ultrastructural organizational relationships, a chemico-morphological relationship can be established with histofluorescence used in conjunction with GDC and GDC identified ultrastructural amine foci with the use of analytical electron microscopy. Thus, this procedure greatly facilitates the perusal of complex areas of the central nervous system in that routine, rapid histofluorescence can identify areas for GDC treatment and ultrastructure evaluation. It remains to be seen which technique has the greater sensitivity, but in the interim such methodologies when applied permit a greater latitude in study during physiological and pharmacology manipulations of animal model systems. (Supported by Salk Foundation of Texas and Grant # USPHS NS 11642.)

841 THE ORIGIN OF MONOAMINERGIC SYNAPSES IN CEREBRAL NEOCORTEX OF IMMATURE RAT: LESION AND ELECTRON MICROSCOPIC STUDIES. Nada R. Zecevic* and Mark E. Molliver (SPON: J.C. Hedreen). Departments of Anatomy and Neurology, The Johns Hopkins University School of Medicine, Baltimore, Md. 21205. U.S.A.

A major innervation of immature rat neocortex by monoaminergic (MA) axons was described by Molliver and Kristt [Neurosci. Lett. 1 (1975) 305]. Previously unrecognized due to their low neurotransmitter levels, the immature MA terminals have a large uptake-storage capacity for catecholamines [Coyle and Molliver, Science 196 (1977) 444]. Monoamine terminals in the CNS can be identified ultrastructurally by the presence of small granular vesicles (SGV), which are the storage sites for catecholamines. In young animals, the blood-brain barrier is incomplete and the visualization of SGVs in central MA terminals can be enhanced by axonal uptake of systemically administered 5-hydroxydopamine (5-OHDA). In the studies cited above, the possibility that non-aminergic terminals were labeled was not excluded; nor were the cells of the origin of these terminals demonstrated.

In order to test the hypothesis that SGV synapses in the lateral neocortex of the infant rat arise from monoaminergic cell bodies in the brain stem we performed three types of experiments; in all three, the distribution of SGV synapses following the administration of 5-OHDA was quantitatively analyzed by an electron microscopic method previously described (cf. Molliver and Kristt, idem.). 1) The neurotoxic amine, 6-OHDA (100 mg/kg), was injected subcutaneously at days 0, 1 and 2. This treatment substantially decreased the number of SGV synapses in lateral cortex compared to their number in the control animals. These results show that the selective destruction of central noradrenergic terminals by 6-OHDA virtually eliminates terminals with the capacity to take up 5-OHDA. 2) the intracerebral injection of 6-OHDA (15 μg/10 μl at age P-2), placed in the mid-brain tegmentum in order to destroy the dorsal CA bundle, significantly lowers the number of SGV synapses in ipsilateral neocortex. 3) a knife lesion in the mid-brain tegmentum also significantly reduces the number of cortical SGV synapses ipsilateral to the lesion. The mechanical lesion controls for the possibility (in #2) of diffusion to the cortex from a local injection of 6-OHDA. These findings confirm that SGV synapses in immature lateral neocortex, concentrated in the primordium of layer IV, are formed primarily by monoaminergic fibers originating from the brain stem. These fibers constitute a major, direct monoaminergic (presumably noradrenergic) projection from brain stem to immature cortex. [Support: USPHS NS-08153, NS-10920 and United Cerebral Palsy Grant R244-71].

842 ASCORBATE BLOCKS AMPHETAMINE-INDUCED TURNING BEHAVIOR IN RATS WITH UNILATERAL NIGRO-STRIATAL LESIONS. John W. Zemp, Lelland Tolbert*, Thomas N. Thomas, and Lawrence D. Middaugh. Dept. of Biochemistry, Medical University of South Carolina, Charleston, S.C. 29403

We have recently reported that ascorbate in physiological concentrations irreversibly inhibits dopamine stimulation of adenyl cyclase in rat corpus striatum in vitro. This report extends this observation to demonstrate an in vivo effect of ascorbate on dopaminergic transmission. Unilateral injection of 6-hydroxydopamine into the substantia nigra produces a degeneration of the dopaminergic neurons with cell bodies in this area. Rats so prepared, when treated with amphetamine, have dopamine released on the side contralateral to the lesion causing pronounced and progressive circling behavior in the direction of the lesioned side six, eight, and ten days after the injection of 6-hydroxydopamine.

Twenty-four Long-Evans rats received unilateral injections of 6-hydroxydopamine (4 μg) into the substantia nigra. The animals were assigned to one of two groups. Six days after lesions were made, all animals were injected with d-amphetamine sulfate (2 mg/kg I.P.). Twenty minutes after injection the number of turns in the direction of the lesion was assessed over a twenty-minute period. The procedure was repeated on day eight with animals in the experimental groups receiving ascorbate (1 g/kg) ten minutes before amphetamine injection while animals in the control group were injected with saline then amphetamine. A final assessment was made with amphetamine only in both groups on day 10. Injection of ascorbate produced a significant decrease in the turning behavior on day eight. No differences between the two groups were found on day six or ten. Ascorbate therefore appears to block dopaminergic transmission in vivo reversibly and may have potential as a therapeutic agent. (Supported by S.C. State Appropriation for Research.)

MOTOR SYSTEMS

843 COMPARATIVE ELECTROPHYSIOLOGICAL STUDY OF CEREBRAL AND PERIPHERAL NERVE PROJECTIONS TO N. INTERPOSITUS AND DENTATUS IN MONKEY.
G.I. Allen, P.F.C. Gilbert*, R. Marini*, W. Schultz*, and T.C.T. Yin. Lab. Neurobiology, Dept. Physiol., State Univ. of New York, Buffalo, New York 14214.

The cerebellum has long been known to receive different cerebral and peripheral inputs over its cortical surface. Because the cerebellar cortex projects onto discrete cerebellar nuclei, which in turn project to different systems ascending toward the cerebrum or descending toward the spinal cord, all apparently involved in the control of movement, it is important to know what cerebral and peripheral signals are integrated by the neurons within n. interpositus and n. dentatus. Several cerebral areas and peripheral nerves were stimulated in cebus monkeys anesthetized with nitrous oxide while recording from individual cerebellar nuclear neurons. The most common inputs to interpositus neurons are from primary motor (MI: 82%), primary somatosensory (SI: 57%), peripheral nerves (53%), premotor cortex in area 6 (PM: 42%), and supplementary motor area of area 6 (SMA: 32%). The most common inputs to dentate neurons are PM (64%), SMA (49%), MI (32%), frontal (30%), and SI (20%), with nerves only influencing 6% of the dentate neurons. The strength of the individual inputs follows the same pattern as the frequencies of these inputs. Superimposed upon these inter-nuclear differences, there is a rostral-caudal gradient. Within both nuclei the neurons located rostrally tend to receive inputs related to the hindlimb (hindlimb MI, SI, and nerves) and from SMA and medial PM. In contrast, the neurons located caudally receive inputs related to forelimb and from lateral PM. Thus, in interpositus the "hindlimb" neurons integrate signals principally from MI (96%), nerves (70%), SI (65%), SMA (35%), and medial PM (24%), whereas the "forelimb" neurons integrate signals from MI (94%), SI (59%), lateral PM (45%), and nerves (36%). Based upon these observations and those from clinical neurology, neuroanatomy, and recordings made in alert, trained monkeys, it is concluded that the lateral cerebellum cooperates with frontal association areas of the cerebral cortex in the planning of movement, whereas the intermediate cerebellum cooperates with sensorimotor cortex and peripheral nerves in up-dating the evolving movement. Furthermore, since the forelimb and hindlimb neurons of the cerebellar nuclei receive inputs that are apparently functionally different, it seems likely that forelimb and hindlimb movements are controlled in different ways.

844 MODE OF PROJECTION FROM A SMALL AREA OF THE MOTOR CORTEX TO THE SPINAL CORD IN THE MONKEY.
H. Asanuma, E. Jankowska*, P. Zarzecki, T. Hongo* and S. Marcus*. The Rockefeller University, New York, NY 10021.

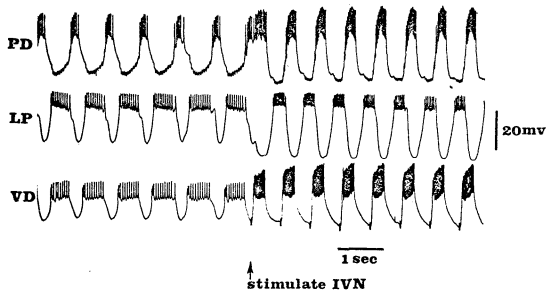
Whether a group of pyramidal tract (PT) cells located close together projects to a given motoneuron pool has been an issue of continuing controversy. We have studied the problem by exploring the pattern of terminal branches of individual PT cells in the spinal cord. Nembutalized monkeys were used. A group of PT cells was recorded simultaneously through the same electrode in the motor cortex while stimulating the lumbar cord through a microelectrode. The lumbar electrode was moved step by step forming a fine grid to detect every branch of a given PT cell group.

It was found that a group of PT cells sent branches to various parts of the lumbar cord. Each PT cell sent branches to motoneuron pools as well as to intermediate regions of the gray matter. In addition to this divergent distribution of branches from a group of PT cells, there was a small area in the ventral horn where all or the majority of the group of cells sent at least one of their branches. The results suggest that when a group of PT cells becomes active, the largest effect appears in a given motoneuron pool with lesser effects in other regions of the spinal cord. (Supported by the NIH grant NS-10705).

845 MONOSYNAPTIC CONTROL OF INTER- AND INTRA-OSCILLATOR COORDINATION OF AN ENDOGENOUS PACEMAKER NETWORK.
Joseph Ayers and Allen I. Selverston, Dept. of Biology, UCSD, La Jolla, Calif. 92093

The pyloric rhythm of the lobster stomatogastric ganglion is driven by a network of three electrically coupled endogenously bursting neurons. The response of this network to periodic monosynaptic inputs was examined in isolated nervous systems. The effect of both EPSP and IPSP inputs depends on the phase at which they occur in the endogenous pacemaker (PD) cycle. Both EPSPs and IPSPs can advance or delay PD subsequent bursts and exhibit qualitatively different phase response curves. The endogenous rhythm can be entrained over a broad range of frequencies by both classes of input. The phase relationships of the discharge of the pacemakers in stimulus cycle are qualitatively different when the cyclic stimulus is slower or faster than the endogenous rhythm for both classes of inputs.

The ventricular dilator neuron (VD) is both inhibited and electrically coupled to the PD pacemaker neurons and its discharge normally alternates with that of the PDs. The inferior ventricular nerve input (IVN) makes monosynaptic EPSP connections with both PD and VD and repetitive trains (ca. 300 msec in duration) in the IVN unit can both entrain the rhythm and shift the phase of VD discharge by 180° so that the VD neuron now bursts synergistically with the PDs (see fig. below). The VD neuron now remains silent between PD bursts. It is concluded that such endogenous pacemaker networks are extremely flexible in their output characteristics for they can both ce coordinated with cyclic monosynaptic inputs and exhibit intra-oscillator phase shifts similar to those observed in more complicated locomotory systems.



846 TEMPORAL RECRUITMENT AND SERVO-PROPERTIES OF PRIMATE MOTOR UNIT RESPONSES TO ANGULAR WRIST DISPLACEMENTS.
P. Bawa* and W. G. Tatton* (SPON.: R.G. Lee). Div. of Physiol., U. of C., Canada.

Imposed angular wrist displacements produce three major, short-latency peaks (termed the M1, M2 and M3 peaks) in the averaged gross EMG activity of the muscles resisting the displacements (Br. Res. 96:108, 1975). Various origins have been suggested for the peaks including synchronised oscillations of motoneuron activity or a series of EPSPs reaching the motoneurons from specific spinal and supraspinal pathways with different loop times.

The subjects (three macaques and four humans) held a handle in a narrow zone. Angular wrist displacements were imposed by step loads produced by a computer controlled torque motor. Up to five different loads (60 gm to 540 gm for monkeys and 0.6 Kg to 6.0 Kg for humans) were presented at random intervals and in random sequence on maintained background "preloads" (up to 180 gm for monkeys and 1.0 Kg for humans). Surface EMG was recorded with silver disc electrodes while single motor units were recorded by "floating", bipolar microelectrodes. Individual motor units were identified and separated using a computer "shape-fitting" program. Average response histograms (ARHs) were constructed for 20 to 75 repetitions of the different step loads. The output was determined from the integrated area of the peaks computed from averaged, rectified gross EMG and from the spike prob./msec of the ARH peaks. Input was taken as the imposed load or as the initial velocity of the displacement. The ARH's showed that 63% of the individual motor units responded significantly (histogram peak includes 95% of spikes occurrences above baseline) over a time course corresponding to only one of the surface-recorded peaks. Two separate unit response peaks were found to occur over the interval of the M1 surface peak (termed M1a and M1b). Twenty-nine % of the units responded over two of the surface peaks. Although the probability of firing increased with increasing loads which ranged from recruitment threshold to the maximal load the monkey could tolerate, the time course of the unit response was maintained. All three surface peaks and the four corresponding motor unit ARH peaks increased monotonically with increasing step loads or initial displacement velocity under all conditions of preload. Human motor units showed responses which corresponded to the surface EMG peaks in a similar manner.

These investigations establish: 1) that the peaks of EMG activity do not merely represent synchronized oscillations in motoneuron activity but result largely from the activation of separately-responding subpopulations of the motoneurons innervating the stretched muscle. 2) that motor unit responses generating the peaks are servo-like in nature. These results will be considered with regard to the size principle of motoneuron recruitment.

847 **PERIOD ANALYSIS OF FAST AND SLOW MUSCLE EMG IN FREELY MOVING CATS.** Bill Betts*, J. L. Smith, and V. R. Edgerton. Dept. of Kinesiology, Brain Res. Institute, Univ. of Calif., Los Angeles, Calif. 90024 U. S. A.

Telemetered EMG from fast-contracting lateral gastrocnemius (LG) and lateral triceps brachii (LT) and slow-contracting soleus (SOL) and anconeus (ANC) in six cats, during unrestrained movement, were sampled at 5000 samples/sec and analysed to detect events defined by maxima and minima which differed in amplitude by more than 120 μv. Intervals between consecutive maxima and minima were computed and expressed as frequencies, while amplitudes were computed between maxima and minima. The reduced data was displayed as a three dimensional histogram.

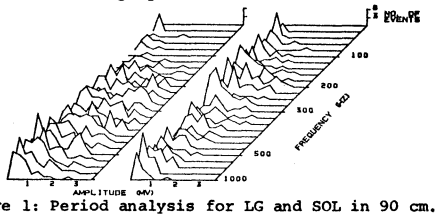


Figure 1: Period analysis for LG and SOL in 90 cm. jumps

During stepping, SOL exhibited a constant median frequency, peak and median amplitude of 180-320 Hz, 2000-3300 μv, and 4-800 μv respectively; while LG was inactive in some steps and exhibited 180-300 Hz, 500-2000 μv, and 3-700 μv for the same parameters in others. The propulsive phase of a series of jumps to a platform placed 30, 45, 60, 75, and 90 cm. above the floor elicited values for SOL similar to stepping; while LG increased in all three parameters during low jumps maintaining a steady peak of 350-750 Hz, 1300-3500 μv, and 650-1500 μv respectively for higher jumps. During treadmill runs, ANC increased slightly between 0.45 and 0.7 m/s in median frequency (250 to 300 Hz) and amplitude (400-650 to 650-900 μv) remaining level at higher speeds (2.0 m/s). The peak amplitude for ANC remained constant throughout the series at 2000-3200 μv. LT maintained steady median frequency, median and peak amplitude at 350 Hz, 400-670 μv, and 1200-2000 μv respectively through 1.1 m/s where it increased to 500-700 Hz, 900-1200 μv, and 2500-3500 μv respectively through 2.0 m/s.

In conclusion, period analysis shows increased recruitment of fast muscle and steady recruitment of slow muscle with increasing treadmill speed and jump height as does integrated EMG but has the advantage of showing both frequency and amplitude parameters. **Supported by USPHS Grant (NS 10423-03).**

848 ROLE OF CENTRAL SEROTONINERGIC MECHANISMS IN AUTOMATIC AND REFLEX SWALLOWING IN RATS. Detlef Bieger, Sch. Basic Med. Sci. and Dept. Physiol. & Biophysics, Univ. of Illinois, Urbana, IL 61801

Rats under urethane anesthesia swallow spontaneously once every 2 to 3 minutes. After acute denervation of larynx and upper trachea, local anesthetization of the oropharyngeal mucosa, and sectioning of all salivary ducts, deglutitive activity persisted in ca. one third of animals. Under such conditions, regardless of the level of basal activity, the serotonin receptor agonists, L-5-HTP and quipazine (QPZ), increased the rate (up to 80 per min) and force of automatic swallowing in a dose-dependent manner over a dose range of 10 to 100 mg/kg and 0.3 to 30 mg/kg (i.v.), respectively. Iproniazid pretreatment (100 mg/kg) enhanced basal activity and shifted the L-5-HTP dose-response curve to a tenfold lower dose range. Harmaline produced analogous effects. QPZ retained its stimulatory activity after pretreatment with iproniazid or with reserpine, which abolished all basal activity.

Selective antagonistic effects were obtained with methysergide and LSD 25, 0.05 to 0.5 mg/kg (i.v.). With administration via the vertebral artery, threshold effects were elicited at agonist and antagonist dosages 8 to 10 fold lower than those required with systemic injections. The effects of either agents persisted after acute precollicular decerebration and those of QPZ were enhanced by bilateral acute vagotomy at the level of the nodose ganglion.

Clonidine stimulated automatic swallowing at doses of 0.05 to 0.15 mg/kg (i.v.). Methysergide antagonized clonidine excitation and unmasked a powerful inhibitory action.

The 5-HT receptor agonists exerted analogous stimulatory effects on reflex swallowing elicited by electrical stimulation of the superior laryngeal nerve. LSD 25 and methysergide counteracted the facilitatory action of the agonists at the same dose levels that antagonized the activation of the swallowing automatism; substantially higher doses of antagonists were necessary to achieve a partial inhibition of the swallowing reflex in the absence of stimulation with agonists.

The data support the hypothesis that the neural apparatus of the swallowing automatism is contained within the pontomedullary region. Serotonin neurons form important links in local neural circuits which operate to set the central excitatory bias for the swallowing 'center' and thus the threshold for its activation by peripheral sensory and suprabulbar inputs. Serotoninergic activation is likely to involve the internuncial neuron network interposed between solitary tract nucleus and effector motoneurons. Supported by the Illinois Department of Mental Health.

849 THE ORGANIZATION OF IA AFFERENT-MOTONEURONAL CONNECTIONS: SOMATOTOPIC ASPECTS AND A NEW HYPOTHESIS. M.D. Binder, J.L. Smith, R.M. Reinking* and D.G. Stuart. Dept. of Physiol., Univ. of Arizona, Coll. of Med., Tucson, AZ 85724.

It has been demonstrated that single Ia afferents from cat medial gastrocnemius (MG) produce EPSPs in >75% of their homonymous motoneurons and in >50% of their heteronymous (lateral gastrocnemius and soleus) motoneurons (Mendell and Henneman. J. Neurophysiol. 34, 171-187, 1971; Scott and Mendell. J. Neurophysiol. 39, 679-692, 1976; Watt et al. J. Neurophysiol. 39, 1375-1392, 1976). In addition to the difference in these projection frequencies, the mean "single fiber" EPSP amplitude is considerably greater in the homonymous cells, strengthening the argument for "species specificity" in Ia afferent-motoneuronal connectivity. Moreover, it has recently been suggested that Ia afferents exhibit a "location", as well as "species specificity" (Scott and Mendell, 1976). As a more stringent test of this latter hypothesis, spike-triggered averaging (STA) was used to measure the distribution and amplitudes of monosynaptic EPSPs produced by single MG Ia afferents in cat MG motoneurons located throughout the rostral-caudal extent of the motoneuronal pool. Following each recording session the point of cord entry of the afferent fiber was traced, and the distance from this point to each motoneuron studied was determined. The motoneurons were divided into two groups; one with locations within 2.5 mm of afferent cord-entry, the other with locations from 2.6-5.0 mm from afferent cord-entry. The projection frequency to the proximal group was 80%, which was slightly higher than the 74% found for the distal group. However, there was no significant difference in the mean EPSP amplitudes of cells in the two groups. In addition, the amplitudes of the EPSPs were not correlated with either motoneuronal or Ia afferent "size" (both measured as axonal conduction velocities). These results suggest that the pattern of Ia afferent connectivity onto homonymous motoneurons cannot be explained solely on the basis of cell proximity. We would propose that Ia afferent connectivity within the homonymous motoneuronal pool displays "functional specificity"; that the "sensory partitioning" which has been shown to exist between motor units and muscle spindles within skeletal muscles (Binder et al. J. Physiol. 257, 325-336, 1976) is complemented by congruent "synaptic partitioning" between spindle afferents and motoneurons within the spinal cord. We would predict that the amplitude of a Ia-motoneuronal EPSP (assessed by STA) is related to the degree of mechanical coupling (quantitated by cross-correlation analysis) between the afferent's receptor and the muscle unit innervated by the motoneuron. (Supported in part by the Fan Kane Foundation and USPHS Grant NS 07888.)

850 ORIGINS OF DESCENDING SPINAL PROJECTIONS FROM THE BRAINSTEM IN MONKEY. A.J. Castiglioni*, M.C. Gallaway* and J.D. Coulter. Marine Biomed. Inst. and Depts. of Physiol. & Biophys. and Psychiat., Univ. of Texas Medical Branch, Galveston, TX 77550.

In 20 monkeys (M. mulatta and M. fascicularis) the cells of origin of brainstem pathways to the spinal cord were identified using the retrograde horseradish peroxidase (HRP) technique. In the vestibular complex, large neurons, scattered in the lateral portion of Deiters' nucleus, were labeled ipsilateral to HRP injections in the lumbosacral cord. Injections of HRP at cervical cord levels labeled neurons in the more medial-dorsal portion of Deiters' and bilaterally in the adjacent medial vestibular nucleus and part of the inferior nucleus. Neurons of the caudal vestibular cell group f were also labeled. At this brainstem level, numbers of cells in the ventral part of the raphe nuclei as well as the large neurons of the medial reticular formation (N. gigantocellularis), mainly ipsilaterally, were labeled from all spinal levels. In the cerebellum, HRP labeled cells were found in the rostral fastigial and adjacent interpositus nuclei contralateral to injections of the upper cervical cord. Scattered large multipolar labeled cells were also located laterally in the brainstem around and dorsal to the facial nucleus where they could be followed anteriorly into pontine levels. Ipsilateral to spinal injections, large neurons of the pontine reticular formation were labeled, mainly in N. pontis caudalis. Laterally, neurons of the nucleus subcoeruleus, adjacent pontine tegmentum, and occasionally of the locus coeruleus proper, were labeled from all spinal cord levels, but were most numerous ipsilateral to HRP injections in the thoracic cord. In the midbrain, the magnocellular red nucleus, in its lateral part, contained neurons labeled from the lumbosacral cord, while neurons towards the medial portion were labeled by injections at higher spinal levels. Labeled neurons were distributed in the midline and adjacent ventral central gray between the trochlear and oculomotor nuclei. HRP injections in the upper cervical cord labeled populations of cells ipsilaterally in the periaqueductal gray, the nucleus of Darkshewitsch, the interstitial nucleus, and the adjacent mesencephalic tegmentum. Groups of labeled neurons were also found, in the deep layers of the superior colliculus and ventrally in the nucleus cuneiformis. Rostral to the red nucleus, a major concentration of labeled neurons was present in the ventral mesencephalic tegmentum. Large multipolar cells were scattered over the central and deep tegmental gray, while smaller elongated neurons were located predominantly in a medial position, such that their axes came to be oriented perpendicular to the third ventricle more rostrally in the medial hypothalamus. Supported by NS 12481.

851 ARE THE "LATE" EMG RESPONSES TO LIMB DISPLACEMENT SERVO-CONTROLLED OR "TRIGGER" RELEASED? C.W.Y. Chan* and R.E.Kearney* (SPON: D.G.D.Watt). Aviat. Med. Res. Unit & Biomed. Eng. Unit, McGill University, Montreal, Canada H3G 1Y6.

Recent findings in humans and primates suggest that the "late" electromyographic (EMG) response to sudden limb displacement, gen-erated with the intention to oppose the stimulus, might be mediated via a long transcortical loop acting in the manner of a servo-system. In the present investigation, we tested this hypothesis by examining the neuromuscular responses elicited in the gastrocnemius (GS) and tibialis anterior (TA) in five normal subjects instructed to resist sudden, servo-controlled RAMP displacements (lasting 500msec) about the ankle joint and comparing them to those elicited by sudden PULSE displacements (lasting 60msec) of the same amplitude and rise time. No significant differences were found in the latency and pattern (shape and area) of EMG response in GS opposing dorsiflexing ramps and pulses; nor in TA opposing plantarflexing ramps and pulses. This lack of difference between responses to two quite different input patterns is not consistent with the behaviour of a response that is servo-controlled. It is however compatible with the concept of the triggered release of a pre-programmed pattern of response. Therefore it is concluded that if a long transcortical pathway is involved, it would probably serve to release preformulated patterns of intended motor control independently of the specific pattern of limb displacement, rather than acting in the manner of a servo-mechanism.

Supported by Canadian MRC.

852 COMPARISON OF SPIKE-TRIGGERED AVERAGES AND STIMULUS-TRIGGERED AVERAGES OF FOREARM MUSCLE ACTIVITY FROM IDENTICAL MOTOR CORTEX SITES IN BEHAVING MONKEYS. P.D. Cheney and E.E. Fetz, Dept. of of Physiology and Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195

In monkeys alternately flexing and extending the wrist against spring-like loads we recorded activity of covarying motor cortex cells and 12 identified forearm muscles (6 flexors and 6 extensors). For some cells spike-triggered averages (SpTA) of rectified EMG activity revealed a transient post-spike facilitation (PSpF) of activity of one or more covarying muscles, probably mediated by corticomotoneuronal (CM) connections of the recorded cell. After documenting the pattern of PSpF produced by a CM cell we stimulated through the microelectrode at the same cortical site with single biphasic pulses during comparable active movements (stimulus duration: .1 or .2 ms; intensity 2-40 μa, usually 5 or 10 μa). Frequency of stimulation was sufficiently low (15/sec or less) to preclude temporal facilitation. Stimulus-triggered averages (StTA) of rectified EMG activity were compiled for the same muscles under comparable conditions at 20 sites where CM cells had produced a distinct pattern of PSpF. Of 83 instances of PSpF of a muscle, all but 6 also showed post-stimulus facilitation (PStF). At 8 cortical sites the relative magnitudes of PStF in each muscle exactly matched the profile of PSpF in the same muscles. At 10 other sites the strongest PStF appeared in the same muscles showing the strongest PSpF. The PStF usually built up much more rapidly than the PSpF, even at stimulus intensities of 5 μa or less. As stimulus intensities were increased above threshold (typically below 3 μa) the relative magnitude of PStF in different muscles remained constant, even though the absolute magnitude increased continuously. At higher stimulus intensities PStF was sometimes followed by suppression. Microstimulation during movements in the opposite direction often produced a post-stimulus suppression in StTA of antagonist muscle activity.

The similarity in pattern of PSpF and PStF supports the conclusion that PSpF is mediated by the recorded cell. The fact that the pattern of PStF was similar to but much stronger than the pattern of PSpF suggests that microstimuli recruited a cluster of CM cells whose terminals are similarly distributed to motoneuron pools. The post-stimulus suppression of antagonist muscles suggests that the spinal connections of the cluster are reciprocally organized.

This research was supported by NIH grants RR00166, NS12542 and NS05489.

853 REVERSALS OF RECRUITMENT ORDER IN MEDIAL GASTROCNEMIUS PRODUCED BY STIMULATION OF DEITERS' NUCLEUS. H.P. Clamann and C.G. Kukulka*. Department of Physiology, Medical College of Virginia, Richmond, Va. 23298.

The responses of single medial gastrocnemius (MG) motoneurons were recorded from their axons in the intact L7 ventral root (VR) of chloralose-anesthetized cats with glass micropipettes. MG motoneurons were identified by their responses to supramaximal stimulation of the MG muscle nerve. Critical firing level (CFL) of an MG motoneuron was determined by stimulating L7 and S1 dorsal roots (DR) with graded single shocks at 1/2 sec while simultaneously measuring the magnitude of the MG reflex response in the muscle nerve, and the response of the motoneuron in L7 VR. CFL was defined as the reflex magnitude at which the unit might or might not respond; if the reflex response was larger, the unit always responded, and if it was smaller, the unit was invariably silent.

A 10 msec train of pulses at 500/sec was applied to the caudal portion of the ipsilateral Deiters' nucleus with stereotaxically placed electrodes. This conditioning stimulus ended 1-2 msec before a single test shock was applied to L7 and S1 DR. Stimulation of Deiters' nucleus increased the monosynaptic reflex response of MG by 100-300%.

60% of MG motoneurons changed their CFL response to stimulation of Deiters' nucleus: cells whose axons had high conduction velocities (> 100 m/sec) showed lower CFL, and slowly conducting motoneurons (< 85 m/sec) showed increases in CFL. The responses of small motoneurons might be due either to inhibition by the conditioning stimulus, or it might have occurred because facilitation of large cells made them relatively easier to recruit.

In several experiments a small motoneuron could be made to respond without fail to L7 and S1 DR stimulation. The addition of a conditioning stimulus from Deiters' nucleus silenced this cell. A component of vestibulospinal input to the MG motoneuron pool appears to be inhibitory to cells of slow conduction velocity.

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854 SPACE-TIME FORM PRINTING BY THE HUMAN CENTRAL NERVOUS SYSTEM. Manfred Clynes. Biocybernetic Institute, La Jolla, California, 92037 and N.S.W. State Conservatorium of Music, Sydney, 2000, Australia.

Repetitive limb movements begun with a single voluntary decision such as tapping, preserve the rate (with some drift). (See e.g., Clynes 1969, in Information Processing in the Nervous System, Ed. K. N. Leibovic, Springer, N.Y., page 186.) But is was not noted before that the particular space-time form of the movement is also preserved without need of further commands and attention. Such a free hand movement may be chosen as a reciprocating straight line motion, oval or circular pattern, or arbitrary free form, and even complex angular movements, and the programmed pattern will maintain itself throughout the repetitions without need of further command and attention. The initial voluntary act which decides the repetition rate also programs the form of the movement. The shape of the movement is preserved by the nervous system.

To produce a subsequent modification of the repetitive pattern, only a single decision is required which takes place in the usual time for voluntary motor action decisions. The pattern modified by such single voluntary action will then continue to maintain itself in the same way as the original pattern.

A single voluntary decision is also required to end the repetitive motion.

Once a person has begun such a "repetitive mode" of a movement form of his choice, he can devote his attention to other activities such as talking, for example, and yet the movement will continue in similar shape while he talks. In changing the pattern however, his attention has to be momentarily focused on the change and talking or other forms of activity may need to be interrupted. This type of generation of repetitive movement, called time-form printing, exists in the frequency range of approx. 5 - 0.4 Hz. It implies a special kind of short term memory. It is amenable to study by average evoked potential techniques.

In spite of the unaware or subconscious nature of the "printing" process, different patterns chosen will tend to influence a person's state of mind differently. Thus a sharp angular movement repeated "mechanically" and a rounded, smooth movement repeated "mechanically" have a different effect on the mental state.

Time-form printing is a "mechanical" carrier phenomenon, whose "non-mechanical" modulation by superimposed decisions provides a basis for a theory of musical rhythm. It appears also to be significant for understanding differential effects of various mantra words in repetitive meditation techniques, the embodiment of the inner pulse of the composer in the musical score, and obsessive compulsive repetitive movement behaviour.

855 IMPAIRED AWARENESS OF ARM POSITION IN PARKINSON PATIENTS IN THE ABSENCE OF VISUAL FEEDBACK. J. D. Cooke, V. B. Brooks, J. Brown and G. Lucier. Depts. of Physiology and of Clin. Neurol. Sci., Univ. of Western Ontario, London, Canada, N6A 5C1.

Studies by Purdon Martin (1) on Parkinsonians and by Hore et al. (2) on monkeys with temporary dysfunction of the globus pallidus have revealed increased dependence on visual information for movement performance. We have studied tracking movements made with and without visual feedback of arm position by two non-medicated patients with idiopathic Parkinson's disease and two age-matched control subjects. Subjects were seated in a chair with their forearm supported on a horizontal handle and they grasped a vertical rod with their hand. The subjects' view of their operant arm was blocked by a sheet. A visual display of target and handle positions was presented on an oscilloscope at the subject's eye level approximately 1m in front of him. Two types of test were used: a) step tracking where the target switched every 2 sec between two positions separated by 32 deg of arc. Targets were not bounded by mechanical stops. b) continuous tracking where the target moved at constant velocity (6.5 or 13 deg/sec) between the same two target positions. After a familiarization period the subject was asked to follow the target movement with, and subsequently, without, the handle position being displayed on the oscilloscope.

Accuracy of arm positioning was assessed from the standard deviations (SD) of the holding positions (step tracking) or of the maximum flexion or extension positions (continuous tracking). Average SDs of arm position from 15-30 trials are given below (patients: BS, BM; controls: WS, JS). Removal of the visual display of handle position increased the variability of arm positioning by a significantly greater amount in the Parkinson patients. In one patient (BS) this increased variability was associated with a gradual drift of arm position towards flexion with no accompanying change in movement amplitude. The other patient (BM) showed an apparent random variation in arm position accompanied by a decrease in movement amplitude which was not significantly different from the control subject.

	STEPS		RAMP (13 deg/s)		RAMP (6.5 deg/s)	
SD(deg)	BS/WS	BM/JS	BS/WS	BM/JS	BS/WS	BM/JS
VISION	2.3/1.9	2.3/2.0	4.0/2.7	2.3/2.4	5.5/2.8	2.4/2.4
NO VISION	3.3/4.5	3.9/2.4	5.9/3.0	4.1/2.8	7.8/2.8	4.8/2.4

(1) J.P. Martin. The Basal Ganglia and Posture. Pitman Medical, London, 1967. (2) J. Hore, J. Meyer-Lohmann & V. B. Brooks, Science 195, 584-586, 1977.
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856 FIRING RATE CHARACTERISTICS OF SMUs IN THE HUMAN MASSETER MUSCLE. B. Derfler* and L.J. Goldberg. Sch. Dent., UCLA, L.A., CA 90024.

Intramuscular recording of spike trains from single motor units (SMUs) was obtained from the masseter muscle of humans while a force transducer positioned between the teeth simultaneously recorded bite-force. Subjects produced 10sec force steps that ranged between 1 and 48 kg. A total of 73 SMUs were triggered and identified for analysis; 41 of these could be followed over 2 or more consecutive force steps. Histograms of interspike intervals (ISIs) were generated for each force level; the mean ISIs ranged from 161-38 ms, and std. deviations (SD) ranged from 47-4 ms. The following observations were made. (1) When units could be triggered over several successive force levels after recruitment, the plots of force vs mean ISI were usually curvilinear and all tended to reach a "plateau phase" of maximum mean ISI, beyond which firing rate remained constant over further force steps. The slope of the curve (rate of change of mean ISI) and position on the Y-axis (indicating minimum and maximum mean ISI) were dependent upon the recruitment threshold of the units. (2) At the recruitment level, most units generated histograms that were asymmetric and positively skewed but as firing rate increased at higher force levels the histograms became progressively more symmetric and narrow, with some reaching even slight negative skew. (3) A plot of SD vs mean ISI was linear with significant positive correlation, except in the vicinity of the shortest mean ISIs where SDs asymptote to values of 4-7 msec. (4) Serial correlation between consecutive ISIs were calculated for 33 sequences of spikes. There were 19 sequences where the respective SMUs had entered their plateau phase of maximum firing rate and these all had negative correlation coefficients with 10 significantly so; the other 14 sequences were not from plateau phases and these had both negative and positive coefficients with none significantly different from 0.

The data can be interpreted according to criteria proposed by Segundo and Perkel (UCLA Forum Med. Sci., 11:349, 1969) as indicating that masseter motoneurons behave: (a) at low drive (threshold) as non-pacemaker cells receiving the equivalent of large, irregular EPSPs; (b) at "optimum drive" (maximum rhythmic firing rate) as non-pacemaker cells receiving the equivalent of many, small, regular or irregular EPSPs (c) at "supra-optimum drive" (strong enough to elicit spikes during the after-hyperolarization) as pacemaker cells receiving the equivalent of large, irregular EPSPs unsynchronized with the rhythmic pacemaker discharge. In addition, we interpret the observations as support for the hypothesis that after-hypolarization summation may provide the natural limit to firing rate for motoneurons in the trigeminal motor nucleus, where no recurrent collaterals have been identified. Supported by USPHS Grant DE 4166.

857 MOTOR OUTPUT RESPONSE TO TRANSIENT DISTURBANCES OF THE HUMAN FOREARM. J. R. Dufresne*, J. F. Soechting and C. A. Terzuolo. Laboratory of Neurophysiology, Medical School, University of Minnesota, Minneapolis, MN 55455.

The dynamic properties of reflex mechanisms responsible for position control of the human forearm were analyzed by relating EMG activity of the biceps and triceps muscles to angular displacement and its derivatives during applied force perturbations. These were produced by a torque motor and consisted of pulses of torque whose amplitude and duration could be precisely controlled. The subjects were instructed to resist the applied perturbations. Either single pulses or a sequence of randomly occurring pulses were used. Modelling of these data using Fourier and cross-correlation analysis showed that the motor output was related primarily to the angular velocity consequent to the applied perturbation (for frequencies above 1 Hz), in agreement with previous results. The gain of this relationship was increased when the applied perturbations were superimposed on a constant level of torque resulting in a high maintained level of agonist activity. EMG output as predicted by modelling closely paralleled the experimental data. The behavior of these relationships during intentional movements was then studied by applying torque pulses while the subject tracked a sinusoid. Thus, reflex behavior could be studied while the biceps acted as agonist or antagonist. For slow movements (below 0.8 Hz), reflex gain is modulated roughly in parallel with the velocity of the movement. For fast movements (2 Hz; above resonance), instead, the reflex gain was maximum while the biceps was silent (being the antagonist and shortened). Throughout the cycle of movement, the gain varied by a factor of 3. These data imply that during fast movements a fusimotor dynamic bias is injected into the antagonist. (Supported by UPHS Grant NS-2567.)

858 BACLOFEN AND VERAPAMIL ON HEREDITARY MUSCULAR DYSTROPHY OF THE CHICKEN. Richard K. Entrikin, Gary T. Patterson*, C. Michael Cisson*, and Barry W. Wilson. Depts., Pharmacology and Avian Sciences, Univ. California, Davis, CA 95616.

In previous studies we found that phenytoin (DPH) improved righting ability and reduced acetylcholinesterase (AChE) activity in posterior latissimus dorsi (PLD) muscles of dystrophic chicks (Science 195:873, 1977), reduced repetitive firing to electrical stimulation in isolated PLD fibers (Fed. Proc. 36:499,1977), and produced a dose-related decrease in AChE activity in chick embryo muscle cultures (Fed. Proc. 36:498,1977). Since DPH affects both myotonia (Neurology 17:359,1967) and calcium metabolism (Science 183:671,1974), other drugs that affect either or both of these systems might also alleviate symptoms of avian dystrophy and decrease AChE activity in cultured muscle cells. We now present results of whole animal and preliminary cell culture experiments with baclofen (Lioresal; CIBA-Geigy) and verapamil (Isoptin; Knoll).

Baclofen (1-10 mg/Kg) was administered in a single i.v., injection (0.2 ml) to seven 25-day-old line 413 dystrophic chicks on two consecutive days. Control dystrophic chicks received saline injection (0.2 ml). Righting ability of each animal was expressed as the exhaustion score (consecutive number of times a chick can right itself from the supine position). Pre-drug all scores were zero. One day after the first injection, all baclofen-treated birds had increased exhaustion scores (range 1-8), and all control birds had scores of zero. After a second injection maximum scores were 10.4 $\pm$ 6.6 (range 4-22).

Single i.v., injections of verapamil (0.2-2.0 mg/Kg) had no effect on exhaustion scores, but chronic verapamil (1 mg/Kg, b.i.d. i.p.) on days 1-25 ex ovo improved scores in 3 of 6 chicks. The PLD and pectoralis major muscles were removed from the two verapamil-treated chicks with the highest exhaustion scores (11, 22). Both histochemical and biochemical determinations revealed decreased AChE activity in these muscles.

Baclofen (0.3-30.0 µg/ml) and verapamil (1-30 µg/ml) were added to 7-day-old cultures of embryonic chick muscle, and 48 hours later cells and medium were assayed for AChE activity. Total activity (Δ cell + Δ medium) was decreased by both verapamil and baclofen (10 and 30 µg/ml).

These results support our theory that antimyotonia drugs (such as baclofen and DPH) will produce acute increases in exhaustion scores of dystrophic chicks and reduce AChE activity of cultured muscle cells. The results suggest that drugs such as verapamil may increase exhaustion scores by a long-term effect on calcium metabolism. (We thank T. Arnold and S. Usoz for assistance. Supported by NIH Grants NS 05308, AM 16716, and the MDA).

859 MOTOR CORTEX UNITS DISCHARGING MOST INTENSELY WITH SMALL PRECISELY CONTROLLED ARM MOVEMENTS ARE MOST SENSITIVE TO SENSORY INPUTS ARISING FROM THE ARM. E. V. Evarts and C. Fromm*. Lab. Neurophysiol., NIMH, Bethesda, MD 20014.

A visual pursuit-tracking paradigm was used for training monkeys to position a handle within a small zone and rotate it by pronation-supination movements. Motor cortex unit discharge was examined in relation to movements carried out in this situation, with particular attention to large (20^0) ballistic movements as compared to small ($\sim 1^0$) precisely controlled movements. Among motor cortex units related to these pronation-supination movements, about half changed discharge frequency only with the large movement, whereas the other half showed changes both with the large and with the small movement. For many units in this latter group, intense discharge was found to occur during even the smallest movements made to achieve accurate positioning of the handle, and such units almost invariably responded (latencies: 20-60 msec) to small handle perturbations which pronated or supinated the forearm. The perturbations, caused by 50-msec torque pulses delivered to the handle, produced handle displacements of about 5^0. Typically, the neurons discharging intensely with precise fine movements were reciprocally related (increase vs. decrease of discharge rate) both to the two directions of movement and also to the two oppositely directed torque pulses. On the other hand, units active with ballistic supination-pronation movements and inactive with small movements were to a large extent uninfluenced by these kinesthetic stimuli. Activity with small movements and high sensitivity to kinesthetic inputs was particularly common in pyramidal tract neurons with more slowly conducting axons, and appears analogous to the high sensitivity to segmental inputs in those spinal cord motoneurons first to be recruited in the course of movement (cf. Henneman, E., Principles governing distribution of sensory input to motor neurons, In: The Neurosiences, Third Study Program, F.O. Schmitt and F.G. Worden (eds.), Cambridge: MIT Press, 1974 (pp. 281-291).

For any particular neuron, the responses to torque pulses delivered during postural stability (i.e., accurate holding of the handle) were compared with the responses to the same torque pulses when delivered immediately before the onset of a ballistic movement, when injected into ongoing ballistic movement, or when applied during precise fine movement. It was found that unit responsiveness to sensory input is relatively enhanced during accurate positioning and controlled small movement, but reduced before and during ballistic movements.

We suggest that sensory feedback continuously modulates motor cortex activity for units controlling precise fine movements and postural stability, while for large ballistic movements the effect of such sensory feedback is relatively attenuated.

860 DIRECT COMPARISON OF RECRUITMENT ORDER WITH NEURAL AND MUSCULAR PROPERTIES OF MOTOR UNITS. Joel S. Faden and Felix F. Zajac. Elect. Eng. Dept., Univ. of Maryland, College Park, Md. 20742.

There is a great deal of controversy regarding the hypothesis that motoneurons innervating a muscle are recruited in an immutable order based on cell size. Confounding this issue is the belief that cell size is correlated with axonal conduction velocity (CV) and contraction time, tension and other properties of the innervated muscle unit. The lack of a unifying solution has been due to the inference of motor unit properties during recruitment studies from a set of assumptions and a combination of experiments. We have been able to measure directly the neural and muscular properties of single units in a given experiment.

In decerebrate cats nerve fibers innervating plantaris (PL) muscle were functionally isolated by dissecting small, intact filaments (30-125μm in diameter; 5-12 mm in length) from the L7 ventral root, to ensure no disturbance of the discharge characteristics of the units as might occur with intracellular electrodes. Axonal CV and tension and EMG produced by the muscle unit to various stimulus trains were recorded using conventional techniques. The intact filaments were then cut distally and the proximal ends carefully preserved. Simultaneous recordings from these proximal filaments containing single PL nerve fibers were obtained during reflexes produced by homonymous muscle afferents to establish their pairwise recruitment order. The stimuli used to elicit reflexes were tendon taps, muscle stretches, repetitive shocks to the muscle nerve and single shocks after post-tetanic potentiation (PTP). PTP was generated by stimulating either the muscle nerve during a distal cold block or dorsal root(s) at 500 Hz for 5-12 seconds at maximum Ia intensity. In all eleven pairs of units studied the unit producing the largest tension, fatiguing the fastest and having the largest CV was recruited after the other unit. Further study of pairs of units showed that the unit in the pair with the largest CV had the largest tetanic tension (60/76), with the reverse (4/76) and with no differences (12/76). These results are consistent with the hypothesis that recruitment order is solely based on motoneuron size, which in turn is highly correlated with the "size" and fatiguability of the innervated muscle unit.

Our sample is perhaps small in order to make definitive judgements, though units defined as type S, FR, and FF are included. The use of other than homonymous input might also change the recruitment order. Further study with a variety of inputs to the motoneuronal pool, such as those occurring during locomotion will contribute to a better understanding of the relevance of the size principle hypothesis. (Supported by NIH grant NS 11518)

861 ORIGINS OF SPINAL PROJECTIONS FROM THE CAUDAL MEDULLA IN MONKEY. M.C. Gallaway*, A.J. Castiglioni*, R.D. Foreman and J.D. Coulter. Marine Biomed. Inst. and Depts. of Physiol. & Biophys. and Psyciat., Univ. of Texas Med. Br., Galveston, Texas 77550.

Retrograde labeling with the enzyme horseradish peroxidase (HRP) was used to identify the origins of descending spinal projections from the caudal medulla in 8 monkeys (M. mulatta and M. fascicularis). In different animals, injections of HRP were made unilaterally into the spinal gray matter of the cervical or lumbosacral enlargement, the thoracic spinal cord, or the upper cervical segments, C1-C3. Beginning at the spinal-medullary junction, medium to large multipolar neurons were found labeled bilaterally in the region of the nucleus supraspinalis, adjacent and just caudal to the decussating fibers of the pyramidal tract. Labeling of these neurons was most abundant following HRP injections in the upper cervical cord and cervical enlargement, but was virtually absent with injections in the lumbosacral cord. More rostrally in the medulla, a major population of labeled neurons was found ventrolaterally in the nucleus retroambiguus. The majority of these labeled neurons were contralateral to HRP injection sites and were labeled from the upper cervical and thoracic cord, as well as from the cervical and lumbosacral spinal enlargements. From the region of the nucleus retroambiguus, labeled neurons could be followed anteriorly where they were situated around and just dorsal to the nucleus reticularis lateralis, and continued forward in small numbers in a position immediately lateral to the inferior olive. At this level, an additional collection of labeled neurons was found dorsally in the lateral medulla beneath the trigeminal complex. These labeled neurons were medium sized, multipolar cells with their long axis directed dorsomedially. The labeling of this lateral medullary zone was heaviest following HRP injections in the upper cervical cord and cervical enlargement. Among other groups of labeled cells in the caudal medulla were scattered neurons in the ventral part of the dorsal column nuclei between the nucleus gracilis and cuneatus. In addition, labeled neurons were located in the caudal nucleus of the tractus solitarius with the heaviest labeling of this area appearing after thoracic spinal injections. The medial medullary reticular formation (nucleus gigantocellularis) and the midline raphe nuclei also contained large numbers of labeled neurons projecting to all levels of the spinal cord. These findings indicate, in the primate, that the caudal medulla contains a number of distinct cell groups which give rise to descending projections to the spinal cord. Presumably these different regions have separate functions in descending sensory and visceral or somatic motor control.

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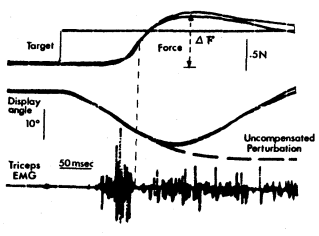
862 ISOMETRIC TRACKING IN THE CAT. C. Ghez and D. Vicario*, The Rockefeller University, New York, NY 10021.

Subjects tracking a rapidly moving target must estimate its trajectory from initial sensory information and scale their motor output to the subsequent position of the target. This study documents such a process in the cat and attempts to characterize the central commands which specify the magnitude of force output generated under isometric conditions.

Cats were trained to apply force isometrically to a strain gauge with their forearm to match a target force level which was stepped at random times. The difference between the target level and the cats' force determined the position of a visual display. Because of inertia and friction in the display device, the step perturbations took 150-200 msec. to be displayed fully (see uncompensated perturbation in figure) and the rate of its motion varied with the size of the step.

The cats responded to these perturbations by rapidly adjusting the force applied. A burst of EMG activity in agonist muscles preceded the force response and lasted until the peak rate of force change (dF/dt). The latency from the target step to this EMG activity was 60-90 msec. After a brief pause, the EMG resumed at a level dependent on the static force exerted. The magnitude of first force change (ΔF) was significantly correlated with the amplitudes of randomly varied target changes despite some overshoot. Both the peak dF/dt and the integrated value of the initial EMG were linearly related to ΔF. By contrast the duration of force change, the time to peak dF/dt and the duration of the initial burst of EMG activity remained essentially constant over the range of forces examined (0.1-2.5 N). We conclude that under isometric conditions the magnitude of intended force may be specified by the magnitude of a pulsatile central command of near constant duration.

The initial agonist "burst" and peak dF/dt specifying the ensuing ΔF are invariably complete before the target shift has been fully displayed. The animals thus extrapolate the full extent of motor output required from the initial parameters of display motion. (Supported by NIH grant 10705).



863 EFFECTS OF AXOTOMY ON NEURAL ACTIVITY DURING LOCOMOTION. J.A. Hoffer, R.B. Stein and Tessa Gordon*. Dept. Physiology, Univ. Alberta, Edmonton T6G 2H7, Canada.

The use of chronic recording electrodes in Silastic cuffs (Hoffer and Marks, Soc. Neurosci. Abstr. 300, 1974; Stein et al, Can. J. Neurol. Sci. 2:235, 1975) has allowed us to follow changes in peripheral nerve activity for long periods of time, prior to and following axotomy. Cuffs were implanted around the sciatic and lateral gastrocnemius -soleus, common peroneal, or posterior tibial nerves of cats. The amplitude and latency of compound action potentials in each branch were measured during stimulation of the sciatic nerve. When these values stabilized some weeks after implantation, the nerve branches were cut distally, and either tied off or sutured to their distal stumps or to nearby muscles which were denervated. Neural activity was recorded during controlled locomotion on a treadmill, and by using cross-correlation techniques the patterns of sensory and motor activity could be separated for each nerve.

Sensory activity declined abruptly following axotomy and did not recover unless reinnervation of sense organs occurred. Even several months after reinnervation, however, sensory activity did not return to control values. Incomplete recovery was accounted for, in part, by a decrease in fiber diameters (as evidenced by declining evoked amplitudes and longer latencies), but was also due to fewer nerve impulses, presumably because some fibers were unsuccessful in regenerating to appropriate sense organs.

Motor activity also declined over the first month following axotomy. This initial decline, greater than that expected from a decrease in fiber diameters alone, was probably attributable to a reduction in synaptic connections onto motoneurons (Mendell et al, J. Physiol. 255:67, 1976). When reinnervation was allowed, motor activity eventually recovered to near control levels, as a result of increasing fiber diameters and probable reformation of synaptic connections. Even when regeneration was prevented by tying off cut nerves, and there was no indication from compound action potentials that fiber diameters had increased, some recovery in motor activity was observed which was maintained for many months.

These findings suggest 1) that the changes undergone by motoneurons in response to axotomy may be partially reversed, even in the absence of reinnervation, and 2) that it may be practicable to record from motor nerves in human amputees and use these signals to control powered artificial limbs.

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864 PATTERNS OF MOTOR-UNIT RECRUITMENT IN A SINGLE FOREARM MUSCLE OF MONKEY IN RELATION TO THE DYNAMICS OF VOLUNTARY CONTRACTION. D.R. Humphrey, M. Rowinski* & A.M. Budacz. Lab. Neurophysiol., Emory Univ. Sch. Med., Atlanta, GA 30322.

To understand better the neural mechanisms that are used in the voluntary control of joint position, we have studied patterns of motor-unit (MU) recruitment in the wrist extensor muscles of monkeys, who were trained to 'track' externally imposed forces and thus maintain a constant wrist position. The animals were required: (i) to generate steady muscle forces of various amplitudes; (ii) to generate triangular or ramp increases and decreases in muscle force, which were of constant amplitude but of variable speed (± 80-1200 g/sec); and (iii) to reposition the wrist following step force disturbances which stretched or shortened the muscle. Intramuscular leads were used to record the activity of small clusters of 3-6 MUs during performance of the various contraction patterns. The units were discriminated on the basis of amplitude, and spike-triggered averaging was used to obtain estimates of the relative amplitudes and durations of their associated twitch tensions. Our major observations were as follows.

(i) During the generation of steady or slowly varying muscle forces, the smallest units in each ensemble were always recruited into activity at lower force levels than were the larger units, and were inhibited last during contractions of the antagonist. These results are fully predictable from the previous work of Henneman and his colleagues.

(ii) When the animal was required to generate triangular force waveforms of constant amplitude but with progressively increasing rates of rise and decay, however, the smaller MUs reached a 'saturation' in their firing rates, so that at the higher rates of tension change their discharge modulated very little. Over this same range, the larger (faster-twitch) MUs exhibited a progressive increase in their firing rate modulation, appearing, at the higher tension rates (700-1200 g/sec), to be largely responsible for the observed modulation in muscle tension. Thus, there may be an order of recruitment from small, slow-twitch to large, fast-twitch MUs in relation to the required speed of a muscle's modulated force output, as well as in relation to the required amplitude.

(iii) MUs of different sizes also responded differently to step perturbations in wrist position. When the muscle was rapidly stretched, the initial, apparently segmental reflex response (latency = 15-25 msec) was most prominent and occurred first in the largest MUs. The response of the smaller MUs was, on the other hand, most prominent during the later period when transcortical reflexes and voluntary adjustments appear to dominate (latency = 60-200 msec). (Supported by NIH Grant NS 10183).

865 CATEGORIZATION AND COMPARISON OF CLINICAL EMG WITH MUSCLE PATHOLOGY BY OPEN BIOPSY EMG. E. R. Isaacs, A. J. Szumski and J. A. Martinez. Depts. Neurol. - Physiol. - Pathol., Med. Coll. Va., Richmond, Va. 23298

Discrepencies between the clinical interpretation of the electromyogram and the muscle biopsy from the same patient prompted the use of an open biopsy EMG technique. The resulting reduction in sampling error permitted more accurate correlations between physiological and anatomical data obtained. One hundred patients ranging in age from 3 to 82 years were selected for muscle biopsy because of suspected muscle pathology. All patients were evaluated neurologically, and with complete electromyographic testing prior to the biopsy. Muscle biopsies were obtained using usual sterile technique under local anesthesia. Muscle tissue was prepared for serial frozen sections and stained by routine histological and histochemical techniques. The electromyograms were categorized descriptively and divided into seven major categories based on motor unit amplitude, duration and potential configuration. Recordings of low amplitude, short duration polyphasic potentials alone, or mixed with normal amplitude, short duration polyphasic potentials showed an eighty-eight percent correlation with histological primary myopathic changes. Twenty one patients had EMGs which displayed a mixture of low amplitude polyphasic potentials and either normal motor unit potentials or normal amplitude but polyphasic potentials. Of these, 80% were associated with selective fiber type atrophy, most of which were Type II fiber atrophy. There was a 90% correlation between large polyphasic motor unit potentials and neurogenic atrophy. Overall, there was a 15% discrepency between the open biopsy EMG and the routine EMG of the opposite extremity. The open biopsy EMG most often correlated with the muscle pathology. It is suggested that selective fiber type atrophy can be reflected in the clinical EMG. Open biopsy EMG provides a reasonable and reliable method of correlating physiological changes with the observed muscle pathology.

866 AERODYNAMIC/TEMPORAL CHARACTERISTICS OF MOTOR SPEECH PRODUCTION IN NORMAL AND APRAXIC SUBJECTS. Thomas W. Jensen* (Spon: D.H. York) Dept. Phys. Med. & Rehab., Sch. Med., Univ. Mo., Columbia, Mo. 65201

Because of the potential neuromuscular diagnostic information that may be contained within the aerodynamic/temporal make-up of motor speech production, a study was designed to tap certain parameters of the normal speech product, and then to apply these data to a clinical population (apraxia of speech). Eighty (80) normal adult females, and two adult females with apraxia of speech, served as subjects for this study. Peak intraoral air pressure and duration of intraoral air pressure, of certain voiceless consonants, were temporally compared to voice termination time and voice onset time preceding and following the consonants of interest. Intraoral air pressure was collected via an open-ended tube placed in the oral cavity. The transduced signal was amplified and displayed on a multichannel recorder. Voice timing data was sensed by placement of a throat microphone on the external neck wall on, or adjacent to the lamina of the thyroid cartilage. The voicing data was amplified and displayed simultaneously with the pressure data. Results of normal subjects revealed consistent aerodynamic/temporal responses to the stimuli presented, within subjects, and across subjects, suggesting that glottal activity is time dependent on supraglottal activity, during speech production. Specifically, peak pressure (expressed in cm H_2O) and pressure duration (expressed in msec.) was dependent upon initiation and release of the intraoral pressure component. To demonstrate clinical application, two subjects, with apraxia of speech, were recorded using the above procedure. Inconsistent intraoral air pressure characteristics were displayed within subjects and across subjects with voice timing not being temporally related to supraglottal activity.

867 INPUTS FROM THE PERIPHERY TO THE MOTOR CORTEX IN THE CAT. K. Larsen*, P. Zarzecki and H. Asanuma. The Rockefeller University, New York, NY 10021.

Neurons in the motor cortex (M-Cx) have small somatosensory receptive fields, but the pathways which convey this sensory input are not known. We have approached the problem by recording cortical evoked potentials following stimulation of superficial (SR) and deep radial (DR) nerves in combination with lesions or cooling of selected spinal tracts and sensory cortex (S-Cx). Following extensive removal of S-Cx, including areas 1, 2, and 3a, the potentials were still evoked in M-Cx, although they were reduced to one-fourth the control amplitude. Cuneate nucleus cooling reduced only slightly the group II response from DR, reduced the SR-evoked response by less than 40%, and eliminated the group I response. Section of either the spinocervical or spinothalamic tract reduced only moderately the magnitude of the evoked potentials in M-Cx. When both tracts were sectioned, however, the M-Cx response was reduced greatly. When spinothalamic tract section was combined with cuneate nucleus cooling, the SR-evoked response was eliminated in S-Cx but remained, although reduced, in M-Cx.

The results altogether indicate that some inputs to M-Cx arrive independently of S-Cx. Some of the group II inputs travel directly to M-Cx through the spinocervical and spinothalamic tracts. The group I input to M-Cx is transmitted through the dorsal columns and probably through area 3a of the sensory cortex.

This research was supported by the NIH grant NS-10705.

868 FIELD POTENTIALS RECORDED FROM THE TRIGEMINAL MOTOR NUCLEUS AFTER LABELING THOSE NEURONS BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. Visaka Limwongse*, Don Rigamonti, and Mark DeSantis. Dept. Anat., Georgetown Univ., Washington, D.C. 20007.

Sprague-Dawley rats received unilateral injections of horseradish peroxidase (HRP; Sigma, type VI; 5% in saline) into either the masseter or the mylohyoid and anterior belly of the digastric muscles. After 24 hr the animals were reanesthetized and electrodes were placed on the nerves to those muscles. With micropipettes, filled with 7% Fast Green in 2M NaCl and having an initial impedance of 1.8-2.2 MΩ, a systematic series of tracks through the ipsilateral trigeminal nuclei were made at intervals of 200 µm. Amplified potentials were recorded at 100 or 200 µm intervals along each track. The animals were sacrificed by perfusion with a buffered aldehyde fixative. Frozen sections of brain were incubated in 3,3' diaminobenzidine containing hydrogen peroxide to identify those cell bodies possessing the exogenous peroxidase. The recording sites were reconstructed from the histological sections containing the HRP labeled motor neurons and the Fast Green marks deposited from the micropipette at known intervals along the depth of electrode tracks.

Field potentials of negative polarity recorded from the trigeminal motor nucleus between 28 and 48 hr after the HRP injection into a muscle did not differ in amplitude, duration or latency from those recorded in uninjected rats. The size of the potential when the nerve to the injected muscle was stimulated was correlated with the distribution of motor neurons containing the HRP. The location of labeled masseter motor neurons and the largest field potentials recorded with stimulation of the nerve to that muscle were in the dorsal and lateral part of the nucleus. The same measures on the motor neuron pool innervating the mylohyoid and anterior belly of the digastric muscles showed them to be situated ventrally and medially in the caudal half of the motor nucleus. This confirms previous morphological studies on the organization of the trigeminal motor neurons in the rat (Mizuno et al., J. Comp. Neurol.,1976; Limwongse and DeSantis, Am. J. Anat., in press). The evidence that trigeminal motor neurons containing exogenous peroxidase conduct action potentials suggests that the simultaneous use of these morphological and electrophysiological techniques may prove a feasible approach to studying other neuronal populations. (Supported by Division of Research Resources, Biomedical Research Support Grant RR05360.)

869 EFFECTS OF UPPER RESPIRATORY TRACT STIMULI ON THE LARYNGEAL MUSCULATURE. G.E. Lucier and B.J. Sessle. Div. Biological Sciences, Fac. Dent., Univ. of Toronto, Canada M5G 1G6.

Although the laryngeal musculature is active in normal protective reflexes such as coughing and swallowing, it is also involved in other paroxysmal respiratory phenomena such as laryngospasm which has been implicated in the sudden infant death syndrome. The purpose of this study was to determine what effect upper respiratory tract stimuli, previously observed by us to have profound effects on respiration and respiratory neurones, especially in kittens, have on laryngeal motoneurones in adult cats. This would also provide data for comparison with effects of similar stimuli in future studies in kittens. The effects of various natural (e.g. saline, water, saturated bicarbonate solution applied retrograde through a laryngeal cannula) and electrical (superior laryngeal, recurrent laryngeal, glossopharyngeal and vagus nerve stimuli) modes of stimulation have been studied in over 80 laryngeal motoneurones. Their activity was monitored by (i) extracellular recording from single neurones in the nucleus ambiguus, (ii) single fibre recording of motoneurone efferents in the recurrent laryngeal nerve, and (iii) single motor unit recording from individual laryngeal muscles. Stimuli (both electrical and natural) which were most effective in modifying laryngeal motoneurone activity were those previously found to be highly successful in altering respiratory neurone activity, thus emphasizing the interrelationship of these two populations of brainstem neurones. Central and single fibre recordings demonstrated that superior laryngeal nerve sensory input is a dominant modifier of laryngeal motoneurone activity. Peripheral motor unit recording in individual laryngeal muscles revealed that superior laryngeal nerve stimuli and especially saturated bicarbonate solution produced, in addition to transient apnea, an inhibition of laryngeal abductor musculature (as recorded from the posterior cricoarytenoid muscle) and simultaneously excitation of laryngeal adductor musculature (as recorded from the thyroarytenoid muscle).

Although these effects reflect preparatory steps in the initiation of protective reflexes such as coughing in the adult animal, they may prove to be augmented in the neonatal animal to produce laryngospasm, accompanied by the prolonged apnea which we previously found to be characteristic of the kitten. Therefore, future studies will examine the effects of these stimuli in kittens.

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870 COMPARISONS BETWEEN HISTOCHEMICAL PROFILES AND CONTRACTILE PROPERTIES OF SELECTED PIGEON MUSCLES. Alfred Maier. Department of Anatomy, University of Alabama, Birmingham, Alabama 35294.

Histochemical profiles were determined and compared to contractile properties in five muscles each of the forearm and leg. The muscles used were chosen for their diversity in fiber type composition, promising likely detection of differences in contractile properties. With the exception of the gastrocnemius, the most frequent fiber seen was a type high in myofibrillar adenosine triphosphatase (ATPase) and oxidative enzyme reaction products, comprising at least one third of the cross sectional area of a muscle. A type resembling the mammalian fast-twitch glycolytic fiber accounted from 11 to 48 per cent of muscle cross-sectional areas. The least frequently observed fiber, except in the gastrocnemius, was a type low in myofibrillar ATPase and moderate in oxidative enzyme reaction products. Of the muscles studied, three in the forearm had none of the latter type while in the leg they accounted for at least 15 per cent of the muscle's cross-sectional area. Leg muscles when stimulated at 5 Hz to give trains of single twitches retained, on the average, at least 85 per cent of their initial tension after 10 minutes of stimulation. In none of the forearm muscles declined the tension below 67 per cent. Tetanic tension/cm^2 bore no relation to the fiber type composition. Muscles of quite divergent histochemical profiles produced similar tensions. Fusion frequencies in muscles lacking fibers low in myofibrillar ATPase ranged from 41 Hz to 60 Hz, whereas in muscles with such fibers they ranged from 32 Hz to 42 Hz. Two muscles of the forearm which contained only fibers high in myofibrillar ATPase had significantly faster ($p<0.01$) time to peak tensions than muscles whose histochemical profiles also included fibers low in myofibrillar ATPase, even if few in numbers. However, the extent of the cross-sectional area composed of fibers which were high in myofibrillar ATPase reaction product showed a poor linear correlation ($r=0.15$) with time to peak tension. A better fit was obtained ($r=0.71$) when comparing the cross-sectional area comprised of fibers low in myofibrillar ATPase reaction product with time to peak tension. The linearity was further improved ($r=0.95$) by removing from consideration muscles with complex fiber distributions such as the gastrocnemius, extensor metacarpi radialis and flexor carpi ulnaris. It was only in muscles in which fiber types were randomly distributed that there was a well recognized trend for the time to peak tension to decrease as the number of fibers low in myofibrillar ATPase reaction product increased. Since complex muscles with restricted fiber type distributions did not altogether follow this pattern, it appears that the number of fibers low in myofibrillar ATPase present is not the only factor influencing time to peak tension.

871 THE ROLE OF STRESS RELAXATION OF MUSCLES IN FORCE DEVELOPMENT. Michael McCormack and Bernardo Dubrovsky. Neurophysiol. Lab., Allan Memorial Institue, McGill University, Montreal, Québec.

During flexion of the legs physical work is done on extensor muscles. This work is stored as elastic energy in the stretched muscles and can, therefore, be re-used. In order to evaluate the time-dependant mechanical properties of muscles, we varied the delay between flexion and extension of the legs of 8 male human subjects required to perform maximal vertical jumps from a force transducer platform, the output of which was registered on a polygraph. In this way the role of stress relaxation of the stretched muscles in force development could be assessed. The delay between flexion and extension of the legs was varied by requiring subjects to 'count from 1 to 5' following their flexion, and then to proceed to the extension (force development) phase of the jump. An objective quantitative estimate of the delay was obtained by analysis of the polygraph output. The delay was operationally defined as the time between the onset of the flexion and the onset of the extension phase. The magnitude of the delay was also varied. In order to more precisely evaluate the role of stress relaxation of muscles, three parameters must be controlled. First, the arms must not contribute to the development of momentum throughout the take-off procedure. This was accomplished by taping the arms of the subjects to their sides. Second, the position of a given subject must be constant in order that the stored elastic energy may be constant. The subject's position could be evaluated through cinematography. Third, it must be established that the force applied in the horizontal axes does not increase with increasing delay. The results showed that subjects raised their center of gravity (height=$\frac{1}{2}$ gt^2) 8-29% higher when the delay between flexion and extension was reduced to a minimum. Similar results have been reported by Asmussen and Bonde-Petersen (Acta Physiol. Scand., 91, 385-392, 1974) who suggested that the storage of elastic energy in muscles might explain the results. Further we find that, for a given subject, the height of jump varied inversely and exponentially with the delay. Interestingly, previous research on isolated muscles has shown that stress relaxation properties of muscles follow an exponential function, the tension decreasing with time (Abbott, B.C., and Lowy, J., Proc. Roy. Soc. London, Ser. B. 146: 280-288, 1957). However, it was found that the total force applied in the horizontal axes increases exponentially in proportion to the magnitude of the flexion - extension delay. We suggest then that the timing between flexion and extension of the legs in performing maximal vertical jumps is crucial to the obtained height of jump and, further, that force distribution is a critical parameter in assessing the importance of elastic storage of energy and stress relaxation in vertical force development.

872 ORGANIZATION OF THE FACE AREA OF MOTOR CORTEX IN MACAQUE MONKEYS. EveLynn McGuinness* and John Allman (SPON: C.B.G. Campbell). Dept. Psychol., Vanderbilt Univ., Nashville, TN 37240 and Div. Biol., California Institute of Technology, Pasadena CA 91125.

Macaque monkeys were surgically implanted with a head restraint bolt and a chamber over the face area of precentral motor cortex using aseptic procedures. After a recovery period, animals were lightly sedated with ketamine HCl and placed in a primate chair with the head immobilized. We stimulated the face region using glass insulated platinum-iridium microelectrodes at current levels of less than 20 µa (0.2 msec pulse duration, 3 msec interpulse interval, 50 msec train of pulses). Experiments were typically conducted 2 or 3 times a week for sessions of 8 to 10 hrs. Electrolytic microlesions were made at appropriate intervals and after a period of about 1 month the animal was killed, its brain sectioned, and electrode tracks reconstructed. Using this technique it was possible to explore the structure of motor cortex in fine detail by advancing the electrode through cortex in steps of 50 or 100 µ. After moving the electrode we waited for several minutes at each site before stimulating to allow the brain to settle. Our results suggest that motor cortex is arranged in fine columns (usually 200-800 µ across). The data were particularly striking in long penetrations parallel to the bank of the central sulcus in which we were able to move tangentially through cortex. As the electrode was advanced in these tangential penetrations, we encountered a series of discrete zones, each of which was related to the movement of a particular muscle in the face. The lowest stimulus threshold points were found in the center of each zone and as the microelectrode progressed toward the edge, thresholds rose slightly until there was a shift to a new muscle movement. The threshold then dropped and the pattern repeated. Typically changes in muscle movement were quite abrupt and successive stimulation points separated by as little as 50 µ could yield different responses. In favorable penetrations thresholds remained under 10 µa, usually ranging from 2 to 6 µa. We very rarely encountered simultaneous movements of more than one muscle when stimulating at threshold level currents. The general organization of the face area was roughly topographical with adjacent muscles clustered together; however, particular muscle movements were sometimes represented in several non-contiguous zones in tangential penetrations. Our data are compatible with the possibilities that these zones are either curving slabs of cortex representing a muscle movement or series of cylindrical columns.

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873 EFFECTS OF COOLING VENTRAL LATERAL THALAMUS (VL) AND SENSORIMOTOR CORTEX ON LONG-LOOP REFLEXES IN MONKEYS. Alan D. Miller and Vernon B. Brooks. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

Perturbations applied to a monkey's forearm can produce three successive EMG responses in upper limb muscles (M1, M2, M3 (1)). M1 (15-35 ms) occurs at the latency of a spinal stretch reflex while M2 (35-70 ms) and M3 (70-100 ms) are thought to depend on supraspinal participation (latencies from our exps). M2 is abolished by postcentral arm region lesions involving areas 1, 2, 3b, most of 3a, and some of 5 (1). Perturbations can produce precentral neuronal discharges at latencies that would permit their participation in the generation of M2 and M3 (2). The 1st precentral response (20-50 ms), preceding M2, does not depend on cerebellar input since it is unaffected by cerebellar nuclear cooling (3, 4). However, the 2nd precentral response (50-100 ms), which precedes M3, but probably occurs too late to appreciably contribute to M2, does depend on cerebellar nuclear function (4).

We examined the possible contribution of a transcerebellar pathway through VL to motor cortex to the generation of M2 and M3 by implanting VL with probes for local reversible cooling in three Cebus monkeys. The arm areas of pre- and postcentral cortex were also cooled for comparison in two of the same monkeys using thermoelectric Peltier modules implanted over the dura. Two monkeys were tranquilized with Atravet, and the arm contralateral to the cooling devices was firmly strapped to a manipulandum handle which was perturbed using a torque motor (5). A third monkey, with VL probe only, was trained to resist perturbations applied to a handle. Biceps EMG responses, obtained with fine-wire intramuscular electrodes, were full-wave rectified and filtered; 25-30 trials were averaged in 5 ms bins with a PDP-12 computer. Cooling VL or sensorimotor cortex delayed and reduced M2 and M3. (Our failure to abolish M2 (cf. (1)) may have been due to insufficient tissue affected by cortical cooling). VL cooling may have depressed M2 by tonic disfacilitation of motor cortex, since phasic discharges of most VL arm area neurons to perturbations occur too late to participate in most of M2 (6). Cortical cooling could have produced tonic as well as phasic disfacilitation of subcortical structures. Pathways through sensorimotor cortex may participate in spinal generation of M2 and M3, with a transcerebellar route via VL contributing mainly to M3.

(1)Tatton et al.Brain Res.1975,96:108-113(2)Conrad et al.Brain Res.1975,94:219-236;Evarts,Science 1973,179:501-503(3)Meyer-Lohmann et al.Brain Res.1975,94:237-251(4)Vilis et al.Brain Res. 1976,117:336-340(5)Cooke & Eastman,Exp.Brain Res.1977,in press (6)Strick, J.Neurophysiol.1976,39:1032-1044.
(Supported by MRC of Canada (PG-1) and USPHS (NS-10311)).

874 MODULATION OF THE SPONTANEOUS ELECTROMYOGRAM (EMG) OF THE TEMPORAL MUSCLE IN THE ADOLESCENT RHESUS MONKEY. Arthur J. Miller, Karin Vargervik*, and George Chierici*. Center for Craniofacial Anomalies, School of Dentistry, University of California, San Francisco, CA 94143.

As part of a long-term study of muscle-bone interaction within the craniofacial region of the rhesus monkey, muscle function has been evaluated by monitoring the spontaneous activity of selected muscles. The temporal muscle as a jaw elevator and posture muscle of the mandible has been studied among these muscles. Spontaneous activity has been defined as that EMG activity exclusive of swallowing and mastication. This activity has been analyzed by one or both methods: 1) by counting the total number of spikes over time in the rectified signal, and 2) by spectral analysis to determine the power distribution of specific frequencies of the background EMG.

The temporal muscle was continuously active in the unanesthesized monkey sitting upright. The mean spike count averaged across 4 monkeys in 16 recording sessions was 160± 14 spikes/sec. Spectral analysis indicated that the mode frequency of the spontaneous EMG was 60 Hz. The spontaneous activity was altered under two short-term conditions which were originally hypothesized to alter the function of the temporal muscle. First, all monkeys were initially anesthesized with a dissociative drug, ketamine HCl (7-10mg/kg, IM). Administration of ketamine HCl increased significantly the power in all frequency bandwidths below 400 Hz within the first 30 minutes but demonstrated the normal distribution by 60 minutes. Secondly, suspension of weights from the mandible decreased significantly the mean number of spikes and the power of the frequency bandwidths below 400 Hz with the larger of two weights (i.e. 150, 300 gm).

The spontaneous activity of the temporal muscle was also evaluated under two long-term alterations. First, detachment of the temporal muscle increased significantly the mean number of spikes of the EMG through the first 24 days but only suggested a slight alteration in the spectral analysis. Comparison of 8 control monkeys with 8 established mouth breathing monkeys (i.e., for 2 years) indicated that the distribution of the spectral analysis histogram did differ significantly. The mouth breathing monkeys had demonstrated morphologically a greater vertical height and a lower jaw position.

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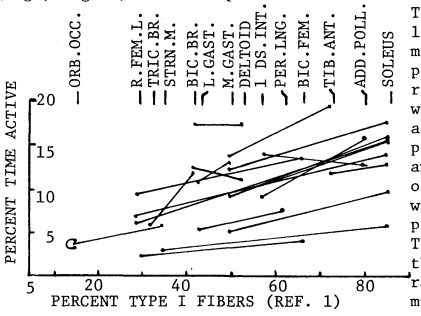
875 CORRELATIONS BETWEEN HISTOCHEMICAL PROPERTIES AND USAGE PATTERNS OF HUMAN SKELETAL MUSCLES. A.W. Monster, H. Chan*, D. O'Connor*. Temple U., Health Sc. Cntr., Philadelphia, Pa. 19140.

Enzyme stains of human skeletal muscle biopsies indicate a number of differences between the metabolic properties of different muscles. There are good reasons to assume that metabolic differences reflect differences in the functional (usage) characteristics of skeletal muscles. These characteristics can be derived from the normal contractile and electrical activity patterns. It is, thus, possible to consider the question: "What aspects of the usage pattern are being expressed by the present histochemical methods?" Day-to-day variability in normal muscle usage as well as population and species differences in the available histochemical data, complicate the analysis of functional-histochemical correlations. In the present study we have tried to overcome the effects of usage variability by: 1) recording simultaneously from two or more functionally related, but histochemically different, muscles and 2) recording activity patterns continuously for day-long time periods using a portable multi-channel tape recorder. Muscular activity was based on the area integral of the rectified electromyogram (EMG). No restrictions were placed on the individuals daily activities but the subject population was somewhat restricted. The EMG recordings showed that a number of muscle usage parameters were highly correlated with the percent type I fibers (e.g., Fig. 1). Muscle pairs are identified by lines in Fig. 1. The gradients of the lines show that the muscles with a higher percent of the (fatigue-resistant) type I fibers were relatively more active. Other usage parameters, such as the amplitude distribution of activity levels, also were correlated with the percent type I fibers. The findings suggest that, taken over a wide range of human skeletal muscles, there is a continuum in the relationship between the usage pattern of a muscle and its histochemical make-up, even though the examined muscles are involved in very different motor activities.



Supported by NS 11574. Ref. 1) Johnson et al. J. Neurol. Sc., 18 (1973), pp. 111-129.

876 ELECTROMYOGRAPHIC RESPONSES TO LOAD PERTURBATIONS IN PARKINSONIANS IN RELATION TO QUANTITATIVE MEASURES OF RIGIDITY AND TREMOR. James A. Mortimer and David D. Webster*. Dept. Neurol., V.A. Hosp. and Univ. Mn., Minneapolis, MN 55417.

The association between quantitative measurements of rigidity and tremor and the normalized magnitude of integrated EMG responses to suddenly-applied load perturbations to the forearm was studied in 17 Parkinsonian patients with varying degrees of clinical rigidity and tremor and in 8 normal subjects. Subjects were instructed to respond to load perturbations in one of four ways: 1) to return the forearm to its initial position as soon as possible, 2) to perform a ballistic flexion movement, 3) to perform a ballistic extension movement, or 4)to relax and allow the forearm to be passively moved. Sixty-four 500 ms. torque steps of 3 Nm. amplitude tending to extend or flex the forearm were presented in a random sequence for each test. Rigidity was measured by integrating the resistance torque as the forearm was passively extended and flexed at a constant velocity by a servo-controlled system.

Positive values of rigidity were associated with tonic stretch responses in both the biceps and triceps muscles. When EMG responses to load perturbations in normal and Parkinsonian subjects were compared, three significant differences were found: 1) Patients with Parkinson's disease had significantly larger responses between 50 and 100 ms. following the load change when the instruction was given to relax. 2) When the intended prime mover in a ballistic movement was stretched, Parkinsonians had a greater discharge than normals between 50 and 75 ms. after a change in load. 3) When the biceps was preloaded and the instruction was given to return to the initial position, normal subjects had a larger EMG response than Parkinsonians between 22 and 50 ms. following the load change. Significant positive correlations were found between the increased EMG activity from 50 to 100 ms. following the load change and quantitative measures of rigidity obtained during relaxation and during performance of a pursuit tracking task. With one exception, arm tremor was uncorrelated with the magnitude of the EMG responses to load changes.

The results add support to the hypothesis of Tatton and Lee that abnormal feedback in long-loop pathways is related to Parkinsonian rigidity by demonstrating a significant correlation between the magnitude of long-latency EMG responses to sudden muscle stretch and quantitative measures of rigidity in an unselected population of patients.

877 CORTICAL AND PERIPHERAL CONTRIBUTIONS TO THE GENESIS AND MODULATION OF THE RHYTHM OF MASTICATION. T. Murakami*, J.P. Lund and S. Rossignol. Fac. Méd. Dent. and Centre Rech. Sci. Neurol., Univ. de Montréal, Canada. (SPON: H.H. Jasper)

Repetitive electrical stimulation (3-500 Hz) of a large area of the anterolateral cortex of the rabbit evokes rhythmical movements resembling normal mastication. However the basic rhythmical pattern of alternating activity in antagonistic muscle groups is generated within the brain stem in response to the cortical input or to certain inputs arising from the mouth (Dellow and Lund, J. Physiol. 215: 1-13, 1971). This present study shows that, as well as acting as activators of the rhythm generator, these inputs are capable of modifying the resulting rhythm.

Stimulation of the upper lip during subthreshold cortical stimulation evokes a long duration burst of activity in the digastric muscle after long latency (100-400 msec). Such a burst may then be followed by a period of rhythmical mastication. When given during masticatory movements, the effect of a stimulus to the lip depends upon the phase of movement in which it occurs. When given during jaw closing and the early part of jaw opening, the cycle time is increased by up to 20%. Conversely, when the shock occurs during the final phase of jaw opening, the cycle is shortened by up to 10%. In the intervening period, it is unchanged.

With low frequency stimulation of the cortex, the mean frequency of mastication (2-5 Hz) is proportional to the frequency of stimulation. In addition, the cycle length is dependent upon the phasic relationship between the stimulus and the movement. When a stimulus is given at maximal jaw opening the cycle length is reduced to 75% of the mean. The gradual displacement of the stimulus away from that point leads to a progressive increase of the cycle length up to a maximum which is 25% longer than the mean. This occurs just before maximal jaw opening.

Stimuli occurring in the critical period at which jaw opening turns into jaw closing have their maximum effect on the cycle length. The displacement of the stimulus pulse by as little as 5% of the total cycle time within this critical period, can halve or double the masticatory cycle length.

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878 CORTICOSPINAL PROJECTIONS FROM THE MEDIAL CEREBRAL HEMISPHERE IN MONKEY. E.A. Murray* and J.D. Coulter. Marine Biomedical Inst. and Depts. of Physiol. & Biophys. and Psychiat., Univ. Texas Medical Br., Galveston, Texas 77550.

The primate corticospinal tract has previously been shown to originate in the primary motor cortex (MI), corresponding to Brodmann's area 4, the first somatic sensory cortex (SI), comprised of areas 3a, 3b, 1 and 2, plus the adjacent area 5, the second somatic sensory region (SII), and the supplementary motor region (MII), corresponding to medial area 6. In addition to these four main functional zones, analysis of the medial hemisphere indicates the existence of an additional, separate, medial posterior parietal cortical region, corresponding to part of area 5 and the adjacent area 7, which contains a topographically organized projection to the spinal cord. Retrograde transport of horseradish peroxidase (HRP) from the spinal cord was used to identify corticospinal neurons in 16 monkeys (M. fascicularis and M. mulatta). With injections of HRP into the cervical enlargement, labeled neurons of cortical layer V were found in the lateral MI, SI and SII regions on the convexity of the hemisphere. On the medial buried aspect of the hemisphere, neurons were labeled in the anterior area 6 on the upper and lower banks of the cingulate sulcus in the supplementary motor region. A second population of labeled neurons was found in the posterior parietal cortex around the posterior end of the cingulate sulcus in Brodmann's area 5. The labeled neurons within this medial part of area 5 were clearly separated anteriorly from the labeled cells in area 6 and laterally from the cells in SI and area 5 on the convexity of the hemisphere. The intervening cortex, comprised of the medial MI and SI regions and immediately adjacent parts of area 6, anteriorly, and area 5, posteriorly was found to contain labeled neurons when injections of HRP were made in the lumbosacral spinal cord. Following injections in the upper cervical cord, labeled neurons in the supplementary motor region extended further anteriorly in area 6 in the medial hemisphere. In the medial posterior parietal zone, labeled neurons were located more posteriorly than those projecting to the cervical enlargement. Labeled neurons extended over the extreme end of the postcentral gyrus, area 5b of C. and O. Vogt, into area 7 on the medial aspect of the hemisphere and in the buried cortex forming the banks of the medial-most part of the intraparietal sulcus. The somatotopic representation in this medial posterior parietal zone thus appears approximately reversed in anterior-posterior orientation to that seen in the rostral supplementary motor region. These findings are consistent with the previous identification of this posterior parietal cortical zone as constituting a "supplementary sensory" region. Supported by NS 12481.

879 REFLEX EFFECTS ON THE DISCHARGE PATTERNS OF GAMMA MOTONEURONES. K.S. Krishna Murthy, James E. Marchand*and Philip L. Gildenberg. Div. Neurosurg. and Dept. Neurobiol. and Anat., Univ. Tex. Med. Sch., Houston, TX 77030.

Variability in the frequency of discharge from gamma motoneurones may increase due to loss of supraspinal control (Ellaway, P.H. and Pascoe, J.E., J. Physiol. 181:200-213, 1965). It is not known, however, whether significant differences exist in the regularity of impulses from gamma motoneurones when activated by various reflex pathways. Recordings have been made from lumbar (L7) ventral rootlets of cats under light sodium pentobarbital anesthesia. Gamma motoneurones were identified from their spontaneous background activity and confirmed from their conduction velocity determined by recording from the same ventral root filament at two points spaced 5-10 mm. (Conduction velocities were in the range of 17-48 m/sec.) Interval histograms were obtained over 2 minute periods with a Nicolet 1072 averaging computer and recorded on paper. The mean and standard deviation of interspike intervals were calculated for further analysis.

Gamma motoneurones were excited by a variety of peripheral stimuli through both segmental and inter-segmental pathways (Gilman, S. and Ebel, H.C., Brain Res. 21:367-384, 1970). Excitation from natural stimulation of ipsilateral proprioceptive afferents improved the regularity. Electrical stimulation of the ipsilateral sural nerve tended to increase the variability of the gamma motoneurones while simultaneously decreasing the mean interspike interval. This observation may have resulted from mixed effects of cutaneous afferents stimulated. It was often found that an initial inhibitory effect on the gamma motoneurone is reversed to an excitation on prolonged electrical stimulation of the sural nerve. Activation through propriospinal reflex pathways, when present, always increased the variability of interspike intervals. Most effective peripheral stimulation appeared to result from pressure applied locally at the joints. It was not unusual to find a single gamma motoneurone being driven to high discharge rates by pressure applied separately at ankle, knee and hip joints. It is suggested that slowly adapting afferents in the various joints may be responsible for this observation since an earlier study (Hunt, C.C. and Paintal, J. Physiol. 143:195-212, 1958) has discounted muscle and cutaneous receptors as a source of excitation for such stimuli.

880 THE ORGANIZATION OF MIDBRAIN-FACIAL SYSTEMS. W. Michael Panneton and George F. Martin, Dept. of Anatomy, The Ohio State University, Columbus, Ohio, 43210.

Since electrical stimulation of the midbrain elicits an array of complex behaviors in the cat and opossum and the facial motor nucleus (FN) is an integral part of many such behaviors, a study was undertaken to reveal the origin, course, and termination of midbrain-facial systems in the North American opossum. Horseradish peroxidase (HRP) placements were made in the FN in order to locate the midbrain neurons which project to it. All of the retrogradely labelled areas, as well as other areas devoid of back-filled neurons, were then submitted to autoradiographic study using tritiated leucine.

Midbrain regions containing HRP back-filling were the ventral periaqueductal gray (VPG), mainly ipsilateral; the contralateral nucleus Darkschewitz (DK); the interstitial nucleus of Cajal (IFLM), primarily rostral portions of the opposite side; and, more caudally near the midbrain-pontine junction, a contralateral group of perilemniscal cells. A few neurons were also labelled in the dorsal periaqueductal gray, but subsequent autoradiographic experiments suggested that their back-filling was the result of HRP spread beyond the facial nucleus into the reticular formation. Leucine injections delivered to the ventral periaqueductal gray and interstitial areas produced label over the medial divisions of the FN bilaterally, especially its caudal auricular and cervical subdivisions. More rostral placements which included the nucleus of Darkschewitsch, produced label over the medial division of the contralateral FN, while the labelling over the homolateral FN was barely above background. Leucine injections into the red nucleus caused light terminal labelling in lateral subdivisions of the contralateral FN. Although injections of more lateral tegmental areas labelled tracts which descend in ventromedial portions of the pontine reticular formation, grains were not found above background over the facial nucleus. Such results suggest that the absence of back-filled neurons in these areas after HRP cases was not artifactual.

In summary both retrograde and orthograde labelling methods have shown that the region ventral to the cerebral aqueduct is the principle source of midbrain-facial connections. This area includes the VPG, the DK and the IFLM. The red nucleus and perilemniscal region provide additional inputs to the FN which appear to be exclusively crossed.

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881 RETICULOSPINAL ACTION ON CERVICAL, THORACIC AND LUMBAR MOTONEURONS. N.G. Pitts, K. Fukushima and B.W. Peterson. Rockefeller Univ., New York, N.Y. 10021.

We have previously shown that neck motoneurons receive monosynaptic excitation and inhibition via a pathway descending from the medullary reticular formation (RF) outside the medial longitudinal fasciculus (MLF). This study was to determine if the same pathway has similar actions on limb and back motoneurons.

Excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) were intracellularly recorded from a diverse group of extensor and flexor motoneurons supplying the forelimb and hindlimb as well as extensor motoneurons supplying the back in cerebellectomized cats anesthetized with chloralose. Motoneurons were identified antidromically and were then studied to determine their responses to 100-200μA, 100μs pulses applied to the brainstem through bipolar concentric electrodes located 1-3 mm below the floor of the fourth ventricle. Arrays of 8-12 electrodes were used to stimulate points ranging from 1 mm posterior to 11 mm anterior to the obex and 3 mm to each side of the midline. Depolarizing current was injected routinely through the recording electrode to facilitate detection of IPSPs. PSPs that began within 0.7 msec after the arrival of the earliest descending volley and that exhibited little temporal facilitation were identified as monosynaptic.

In 28/38 hindlimb motoneurons monosynaptic excitation was observed following stimulation of the MLF. Monosynaptic EPSPs were also seen when the medial pontine RF was stimulated. Only 2 of 25 hindlimb motoneurons gave monosynaptic EPSPs to medullary RF stimulation and we generally failed to see any response to single pulses. Forelimb motoneurons behaved similarly: 27 of 34 exhibited monosynaptic EPSPs following stimulation of the MLF or medial pontine RF. Only 1 of 32 cells was monosynaptically excited by medullary RF stimulation. No monosynaptic IPSPs were evoked in limb motoneurons by RF stimulation.

Monosynaptic EPSPs were seen in 30 of 34 back motoneurons following stimulation of the MLF or pontine RF and in 10 of 32 motoneurons following medullary RF stimulation. Back motoneurons (13 of 43 cells) also received inhibition from this RF region which required multiple shocks and had a much longer latency suggesting that the inhibition descends primarily via a polysynaptic pathway.

These findings suggest that reticulospinal fibers originating in the medulla act on axial motoneurons at all levels but seldom on limb motoneurons. Therefore, the excitatory and inhibitory actions from the medulla are distinct from the excitatory projections that originate in the pontine RF and act on both limb and axial motoneurons. Supported in part by Grants NSF 75 00487 and NIH NS 02619.

882 PROPERTIES OF THE MOTOR PROGRAMS UNDERLYING VISUALLY TRIGGERED ARM MOVEMENTS IN MONKEYS. Andres Polit* and Emilio Bizzi. Dept. Psychol., M.I.T., Cambridge, MA. 02139.

These experiments were addressed to the problem of elucidating the organization of the nervous system underlying the performance of a simple motor act. Our basic preparation was a monkey trained to point at a target light. The monkey sat in a primate chair with its forearm fastened to a manipulandum which permitted flection and extension of the forearm about the elbow in the horizontal plane. The target lights were mounted on a 14" arc centered at the elbow and spaced at 5 degree intervals. The monkey was required to position its forearm within a 10-15 degree target zone centered about the lights, and hold a position for about 1 second. Four adult monkeys (Macaca mulatta) were used.

We tested their performance prior to and after bilateral dorsal rhizotomy (C2-T3). Movements were performed without the sight of the arm both before and after the surgical intervention. Consequently, after deafferentation the movements were performed "open loop". Remarkably, within five days postoperatively, the animals were able to perform the previously learned task.

In the first series of experiments we unexpectedly displaced the arm within the reaction time of the monkey, and observed the outcome of this displacement on movement termination. Our results indicated that in spite of the presence of unexpected proprioceptive responses, the arm moved accurately to the target. The same procedure was used in the deafferented monkeys, yielding qualitatively the same results, i.e., a displacement of the initial position did not affect the attainment of the intended final position. The electromyographic responses were carefully examined in the postoperative state to ascertain the lack of any short latency changes in EMG following the unexpected displacement of the arm.

These results are relevant to the question of what is being controlled by motor commands. It appears that the controlled variable is an equilibrium point resulting from the interaction of agonist and antagonist muscles. Consequently, a change in the equilibrium leads to movement. This view allows one to consider movement and the attainment of a posture as a result of a single process.

883 METABOLIC CHANGES IN RESPONSE TO STIMULATION OF SINGLE FROG MUSCLE FIBERS IN WHICH CONTRACTION IS UNCOUPLED FROM EXCITATION BY STRETCH AND HYPERTONIC SOLUTION. S. I. Rapoport, V. Nassar-Gentina* and J. V. Passonneau. Lab. Neurophysiology, NIMH and Lab. Neurochemistry, NINCDS, Bethesda, MD 20014.

At 15 °C, stretch of isolated single fibers of the semitendinosus muscle of R. pipiens, to sarcomere lengths (SL) of 3.8 μ or 4.7 μ from a rest SL of 2.3 μ, did not alter concentrations of PCr, ATP, glucose-6-P or glycogen. Stimulation of fibers at a SL of 2.3 μ, for 150 sec and at a frequency of 20 Hz, reduced PCr by almost 100% and ATP by one-third, elevated glucose-6-P and did not alter glycogen concentration. Fatigue was produced, and was evidenced by a reduced mechanical response to a 200-msec tetanus following the 150-sec stimulus train. Stimulation for 150 sec of fibers at a SL of 4.7 μ produced no tension, yet reduced PCr by 70%. ATP was unaltered and G-6-P was elevated. Tetanic stimulation of fibers at a SL of 3.8 μ produced contractile and metabolic changes between those at 2.3 μ and 4.7 μ, as well as fatigue. As fibers stretched to a SL of 4.7 μ consumed PCr but did not generate tension during stimulation, a large fraction of PCr consumption in stretched fibers was unrelated to force generation. On the other hand, stimulation of fibers in which excitation-contraction was abolished by a hypertonic soak resulted in no observed metabolic changes nor in fatigue. The soak by itself increased base line tension and PCr consumption. The findings indicate that stretch uncouples contraction from excitation differently than does a hypertonic soak, at a later step that involves reduction of actin-myosin overlap. PCr consumption in stimulated, stretched fibers may be required for reuptake of calcium that is released by excitation, and calcium may be released to a lesser extent when fibers are stimulated in a hypertonic soak. Furthermore, as fatigue occurs in fibers with an approximately normal ATP concentration, fatigue probably is due to failure of excitation-contraction coupling.

884 A MORPHOLOGICAL DESCRIPTION OF MOTONEURONS IN THE UPPER CERVICAL SPINAL CORD OF THE ADULT CAT. P.K. Rose and F.J.R. Richmond*. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Most descriptions of motoneuron morphology are based on Golgi stained neurons found in the cervical and lumbosacral enlargements of young animals. Little is known, however, about the organization of motoneurons in the upper cervical spinal cord. In the present experiments therefore, intracellular injection of horseradish peroxidase (Snow, Brown, and Rose, 1976) was used to examine the morphology of electrophysiologically identified motoneurons in the upper cervical spinal cord of the adult cat. Cats were anaesthetised with chloralose (60 mg/kg) and paralysed with gallamine triethiodide. Motoneurons were identified by antidromic action potentials elicited by stimulation of dorsal neck muscle nerves. Other neurons, not antidromically excited, were also stained and frequently these neurons were found to have axon projections to the ventral root. These neurons were included in the population of motoneurons to be described.

The somas of motoneurons in the upper cervical spinal cord were usually found in Rexed's lamina IX, but were not restricted to this region and several were located in the nucleus commissuralis on the medial wall of the ventral horn. Axons were darkly stained and usually followed a simple, direct trajectory to the ventral root. Several motoneurons also projected to laminae VIII and IX via axon collaterals.

Dendritic trees of motoneurons in the upper cervical spinal cord were substantially larger than those of motoneurons located in the lumbosacral enlargement and stained with Procion Yellow (Barrett and Crill, 1974). The rostral caudal extent of dendritic trees ranged from 2150 μm to 3500 μm. Dorsally directed dendrites usually spread to lamina VII. Two motoneurons had dendrites which crossed into the contralateral spinal cord via the ventral commissure. All motoneurons had fine dendrites which extended into the lateral and ventral funiculi. Distinct swellings were frequently seen along these dendrites and at their termination.

These results suggest that motoneurons found in the upper cervical spinal cord differ morphologically from those found in the lumbosacral spinal cord (Barrett and Crill, 1974). Whether this difference arises from the different technique used to stain the motoneurons or is related to the different connections of upper cervical spinal cord motoneurons must now be determined.

Supported by the Canadian Medical Research Council.

Barrett, J.N., and Crill, W.E. (1974). J. Physiol. 239: 301-324.
Snow, P.J., Rose, P.K., and Brown, A.G. (1976). Science 191: 312-313.

885 THE CONTROL OF CROSSED EXTENSOR AND CROSSED FLEXOR RESPONSES. S. Rossignol. Ctr. de Recherche en Sciences Neurologiques, Faculté de Médecine, Dept. Physiologie, U. de Montréal, Québec, Canada, H3C 3T8.

Painful skin stimuli of a hindlimb evoke long latency, long duration contractions of ipsilateral flexor muscles in acute low spinal cats pretreated with Clonidine, a noradrenergic stimulant. In the contralateral limb however, contractions occur in the extensors if the limb is initially flexed or in the flexors if initially extended (S. Grillner and S. Rossignol, IIId Int. Symp. Motor Control, Bulgaria, Abs. 28, 1976). What is the contribution of the contralateral limb afferents to this reflex reversal? A progressive dorsal rhizotomy of the contralateral side was performed from S1 to L4 inclusively. Section of S1 did not alter the phenomenon. Section of L7 abolished almost completely the flexor responses when the limb was kept extended. However with the limb in flexion, extensor responses were not only present but increased. This pattern was further enhanced by cutting L6. Following section of L5, large extensor responses appeared both with the limb in flexion or in extension. This remained after cutting L4. Thus crossed extensor responses in absence of peripheral afferents are not only present but are larger both in amplitude and duration. Preparations with weak or absent crossed extensor responses developed large extensor responses after deafferentation. Crossed flexor responses, on the other hand, were never observed after deafferentation and thus their occurrence depends on peripheral afferents. Preliminary results in cats with intact dorsal roots indicate indeed that, with the limb in flexion, a position that would favor extensor responses, stretching the cut tendon of an ankle flexor (tibialis anterior) inhibits extensor responses and leads to flexor responses. With the limb in extension, however, stretching of the ankle extensor Gastrocnemius does not block the flexor response. It is concluded that when the limb is kept in extension the stretch of flexor muscles causes an inhibition of the crossed extensor responses and a facilitation of crossed flexor responses. On the contrary, when the limb is flexed thus removing the stretch of flexor muscles crossed extensor responses appear. Such peripheral control of crossed limb reflexes may be useful during locomotion. During most parts of a step cycle, the position of the limb is such that crossed extension could be used to support weight during a contralateral flexion. However at the end of the stance phase, when the limb is being fully extended and the flexor muscles stretched a crossed flexor response preceding extension would be more appropriate. Peripheral afferents would then be essential to signal such condition during the step cycle.

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886 TRANSCORTICAL FACILITATION OF THE H- (MONOSYNAPTIC) REFLEX IN MONKEYS. D. G. Rüegg*, J.-M. Lachat* and M. Wiesendanger. Institut de Physiologie, Pérolles, CH-1700 Fribourg, Switzerland.

The recovery curve of the monosynaptic spinal reflex (H-reflex) is characterized by an early and a late facilitation which is superimposed on a long-lasting inhibition. The mechanism of the late facilitation is not known. The hypothesis was tested that a transcortical loop for low threshold muscle afferents could be responsible for the late facilitation.

Four monkeys were trained, with a leg fixed in a fiberglass cast, to maintain a prescribed background activity in the soleus muscle. Under these conditions H-reflexes of large amplitude were obtained with little variability. A conditioning stimulus was used which was just subthreshold for the H-reflex whereas the test stimulus was adjusted to elicit H-reflexes without contamination by a direct motor response (M-wave). We found that the late facilitation started at a latency of 50 to 60 msec and reached a maximum at about 100 msec following the conditioning stimulus.

Blocking of the motor cortex by reversible cooling or an irreversible lesion abolished the late facilitation without changing the early facilitation and the long-lasting inhibition. A transcortical pathway was also indicated by the following latency measurements: a cortical potential was evoked in the somatosensory cortex at a latency of 15 msec, and in the motor cortex at a latency of 25 msec. Motor responses in the soleus muscle occurred 25 msec following stimulation of the motor cortex. The sum of these latencies was compatible with the onset of the late facilitation and the long-lasting inhibition.

We conclude that the late facilitation produced by stimulation of the low threshold muscle afferents is mediated via the motor cortex.

887 MECHANISMS OF THE CLASP-KNIFE REFLEX. W.Z. Rymer, J.C. Houk and P.E. Crago. Dept. Physiol., The Johns Hopkins Univ. Sch. of Med., Baltimore, Md. 21205. (Supported by NIH06828 and NIH57240).

The clasp-knife reflex is the abrupt yield in force provoked by flexion of a spastic limb. Although classically attributed to tendon organs, it has recently been ascribed to the inhibitory actions of secondary spindle receptor afferent fibers (1). However, several key issues remain unresolved. Specifically the autogenetic basis of this reflex has not been demonstrated directly, the apparent inhibition has not been adequately distinguished from withdrawal of excitation, and the receptor basis has not been established with fiber recordings. We used a model of the clasp-knife reflex provided by stretch of the soleus muscle in the mid-collicular decerebrate partly spinalized cat (2). The soleus muscle was subjected to ramp stretch of varying amplitude, initiated at several different muscle lengths. Measured variables included muscle length, tension and EMG, together with the discharge of single muscle afferent fibers, teased from dissected small dorsal root filaments. The dorsal roots were otherwise intact. In 5 preparations, dorsal hemisection of the thoracic cord converted a classical soleus stretch-reflex response to a clasp-knife pattern. Specifically, when soleus stretch exceeded some threshold length (typically near -10mm), the initial stretch-induced excitation was converted to inhibition. The degree of this inhibition was most clearly related to absolute muscle length, rather than incremental length change. When muscle stretch was superimposed upon the tonic vibration reflex, the inhibition was reproduced, thus the effects are unlikely to have resulted from withdrawal of Ia afferent excitation.

Although the EMG declined as a function of absolute muscle length, the decrement in EMG was also correlated with initial and peak force levels, behavior not consistent with observed secondary spindle receptor action. Furthermore, a profound inhibition of EMG and force was produced by gentle manipulation of the tendon and muscle aponeurosis. In a limited preliminary examination we isolated several slowly conducting nerve afferents which were not derived from spindle or tendon organ receptors but which responded to muscle manipulation and maximal muscle stretch.

The generation of the clasp-knife reflex in a separated soleus, within an otherwise denervated limb verifies the autogenetic basis of this response. However, the dependence of this effect on initial force levels together with its' prolonged time course are not in keeping with either secondary ending or tendon organ action. It is suggested, tentatively that the clasp-knife reflex is the outcome of stretch induced activation of free nerve endings, presumably of groups II and III diameter.

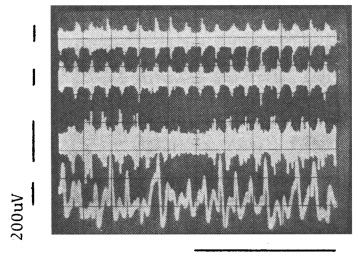
(1) Burke, D., Gillies, J.D. & Lance, J.W. (1970) J. Neurol., Neurosurgery, Psychiatry 35, 216-223.
(2) Burke, D., Knowles, L., Andrews, C. & Ashby, P. (1972) Brain 95, 31-48.

888 EXCITATION AND INHIBITION OF PROXIMAL MUSCULATURE BY MICROSTIMULATION OF PRECENTRAL CORTEX. Edward M. Schmidt and Joan S. McIntosh. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Chronic recording intracortical microelectrodes implanted in the precentral gyrus of monkeys were used for single cell recording and microstimulation. The electrodes were implanted to a depth of 1.5mm (approximately layer V). Single unit activity from the same neuron was recorded before and after microstimulation with trains (5 to 35 pulses, 400Hz) of capacitively coupled 0.2msec pulses up to current levels of 20μa, indicating that stimulation did not permanently damage neurons near the electrode. Both excitation and inhibition have been produced in different muscles, revealed by EMG activity, from the same cortical site. Inhibition was more difficult to demonstrate unless the inhibited muscle was tonically active prior to stimulation or a rebound excitation occured at the end of the stimulus train. Both proximal as well as distal musculature can be activated in the awake monkey with the stimulation parameters we have employed. Either prior to or after microstimulation, through a specific microelectrode, single cell operant conditioning techniques were employed to modify the firing rate of the recorded cell. The movements made by the monkey in controlling the cell firing rate were usually associated with the muscle that was activated by microstimulation. When the cortical cell could be activated by peripheral stimulation, such as stroking the hair and skin overlying the external oblique m, microstimulation produced excitation of this muscle. These findings indicate a somatotopically organized input-output relation.

889 NATURALLY-OCCURRING FACIAL "TREMOR" SYNCHRONIZED WITH RHYTHMICAL THALAMIC NEURONAL ACTIVITY IN RATS. K. Semba*, H. Szechtman*, and B. Komisaruk. Inst. Anim. Behav., Rutgers U., Newark, NJ 07102

A fine (approx. 9 c/sec) passive tremor of the jaw and/or vibrissae was observed in normal rats when they stood still after being placed into a novel environment. The tremor was distinctly different in frequency, intensity, and behavioral context, from movements involved in gnawing, tooth chattering, or exploratory sniffing. Individual tremor movements (recorded as EMG) occurred in synchrony with individual bursts of multiunit activity (MUA) recorded in the ventrobasal complex of the thalamus and with individual "spikes" in the cortical (frontal-occipital) EEG (see figure). Single trains of this rhythmical activity often lasted more than a minute. The phase relationships between EMG and MUA differed among individuals, but tended to remain consistent within each individual. Movement artifacts were discounted since 1) the moment of occurrence of individual tremor movements and MUA bursts were interdigitated rather than simultaneous, and 2) during high amplitude EMG bursts accompanying sniffing (associated with EEG theta rhythm), tooth chattering, eating or licking, no corresponding activity in the MUA was observed. We also discounted the possibility that the neural activity was generated by reafference, for 1) during vigorous non-tremor sniffing movements of the vibrissae, or chattering or chewing movements of the jaws, the rhythmical MUA was absent (although the units did discharge if the vibrissae contacted an obstacle or were brushed by the experimenter), 2) rhythmical MUA often continued both during brief pauses in the motor tremor, and in its absence, 3) injection of Xylocaine subcu into the face abolished sensory responses of the thalamic units, but the rhythmical MUA persisted. Since this rhythmical neuronal activity is apparently not simply a sensory correlate of the tremor, it may be involved in its central production, and is therefore potentially of value in understanding Parkinsonism.



890 DISCHARGE CORRELATES OF FTG UNITS DURING SPONTANEOUS BEHAVIORS. J. M. Siegel, D. J. McGinty and S. M. Breedlove*. V. A. Hospital, Sepulveda, CA 91343 and University of California, Los Angeles, CA 90024.

We have previously reported that discharge in pontine gigantocellular tegmental field (FTG) neurons recorded in unrestrained cats is closely related to motor activity (Science 196: 678-680, 1977). We now report further observations on the activity of these cells during a variety of behavioral states. Cell discharge was monitored during quiet immobility, alert immobility, eating, grooming, lapping, exploration and sleep.

FTG discharge did not relate to any specific behavioral state, but rather related to directionally specific movements which could occur during a variety of behaviors. Of 45 units tested, 30 were related to head and neck movements, 4 to ear movements, 1 to forepaw movements, 3 to tongue movements and 4 to facial movements. FTG discharge was not correlated with directionally specific eye movements observed during head restraint. Units showed no evidence of habituation during repetitive motor activities. Most FTG units discharged during REM sleep, a time of intense internal motor system activation. Unit discharge could often be seen to occur in conjunction with the muscle twitches of REM sleep. We saw no relationship between FTG firing and level of arousal or alertness. Movement correlations seen during grooming activity were the same as those seen during play or attack behavior.

Sensory stimuli often elicited unit discharge whose intensity was correlated with obvious motor responses provoked by the stimuli. Forced acceleration of the cat's head produced intense discharge in many FTG units. However, the maximum response was associated with induced head acceleration in a direction opposite to that which produced maximum unit firing during spontaneous head movements, i.e., a unit which responded maximally when the cat made spontaneous head movements to the right would fire preferentially when the experimenter twisted the cat's head to the left. Response to repeated head movement was variable with irregularities in response correlated not with the velocity of head movement, but rather with the cat's active resistance to the manipulation. During complete head restraint unit discharge remained correlated with specific motor activities associated with struggling.

These results support our previous findings that in the unrestrained cat FTG discharge is closely related to specific motor activities. A film of FTG unit discharge in the unrestrained, behaving cat will be shown.

891 THE ACTIVITY OF NEURONS IN THE SUPPLEMENTARY MOTOR AREA DURING A MAINTAINED PRECISION GRIP. Allan M. Smith. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Québec, Canada.

Monkeys were trained to grasp a hand-held force transducer and maintain specified pressures between the thumb and forefinger for a one-second duration ("the maintained precision grip"). The activity of single neurons was recorded in the hand region of the supplementary motor area (SMA) described by Woolsey et al. 1950. Although microstimulation (300Hz. for 300msec. up to 50uA.) through the recording electrode failed to evoke twitches in the muscles of the wrist and fingers, 60 neurons were found that showed reliable changes in activity related to performance of the precision grip. These units were located in the medial convexity of area 6 between the tail area of primary motor cortex and the face area of the SMA in agreement with the original description given by Woolsey et al. (1950). Many of these neurons were extremely sensitive to light touch and the receptive fields often covered the entire arm from the shoulder to the fingers. Some SMA neurons in the hand area discharge prior to EMG activity recorded in the flexor and extensor muscles of the wrist and fingers. The discharge patterns of SMA neurons appear, on initial analysis, to resemble the discharge patterns of neurons in the hand region of area 4 (Smith et al. 1975). The majority of units were more active during the dynamic phase of force application as opposed to the period of static maintained force. The activity of the flexor and extensor muscles of the wrist and fingers is also correspondingly greater during this period. The preliminary results suggest that a substantial number of neurons in the supplementary motor area are involved in the motor function of the hand.

Supported by the Medical Research Council of Canada.

892 CROSS-CORRELATION ANALYSIS OF EXTENSOR EMG'S AND HAND TREMORS FROM NORMAL AND PARKINSONIAN SUBJECTS. Robert N. Stiles and Robert S. Pozos. Dept. of Physiol. and Biophysics, Univ. Tenn. Cntr. Hlth. Sci., Memphis, TN, 38163.

Cross-covariance analysis was performed on simultaneously-obtained 16-sec records of wrist extensor EMG's and postural hand tremor oscillations from normal and parkinsonian subjects. Bipolar, surface EMG's from the extensor digitorum communis muscle were digitized at 1024/sec, full-wave rectified and smoothed, resulting in an amplitude demodulated EMG signal with equivalent sampling rate of 64/sec. Hand tremor was detected with an AVR-250 accelerometer mounted at 16 cm from the wrist, and the voltage analog of this acceleration was digitized at 64/sec (Stiles and Pozos, J. Appl. Physiol. 40: 990-998, 1976). The cross-correlation (time domain) and coherence (frequency domain) functions were calculated for values of root-mean-square (rms) displacement amplitude of the tremor that ranged between about 30 and 30,000 μm. (These amplitude levels of tremor from normal subjects occurred as each subject maintained the hand extended horizontally for periods of 15-45 min.) Results obtained for both groups of subjects indicate that, for rms displacement levels above about 100 μm, the amplitude demodulated EMG's are highly correlated with the tremor (at the tremor frequency), with coherence values generally between 0.6 and 0.8. For displacement levels below about 100 μm, the coherence between these two variables decreased to values as small as 0.2 at rms displacements of 20-30 μm.

These data are consistent with the hypothesis that the large-displacement postural hand tremor of parkinsonian subjects is an exaggerated normal tremor. The relatively high correlation between the demodulated extensor EMG and hand tremor having rms displacements greater than about 100 μm suggests that additional cross-spectral analysis, resulting in values of gain and phase between these two variables, may be warranted. (Supported in part by USPHS Grants HE-05612 and NS-08692).

893 INPUT TO PRIMATE MOTOR CORTEX FROM POSTERIOR PARIETAL CORTEX. P.L. Strick(1), P. Zarzecki(2), and H. Asanuma(2). (1) Veterans Administration Hospital, and Depts. of Neurosurg. and Physiol., Upstate Med. Ctr., SUNY, Syracuse, NY 13210; (2) The Rockefeller Univ., New York, NY 10021.

The characteristics of some of the neurons in the posterior parietal cortex (areas 5 and 7) suggest that this region may be involved in the control of limb movement (Mountcastle et al, 1975). Using anatomical and physiological methods, we now demonstrate a direct projection from area 5 to the motor cortex (area 4). Small injections of HRP were restricted to the gray matter of the motor cortex 'arm area'. Retrogradely labeled neurons were found in the anterior bank of the intraparietal sulcus in area 5. These neurons occurred in layer III. They were largely pyramidal shaped and 10-30 μm in diameter. The injections labeled at least as many, or more, neurons in area 5 as in the primary somatic sensory cortex. HRP injections placed more laterally in the 'arm area' labeled neurons more laterally in the intraparietal sulcus.

Physiological experiments were performed in unanesthetized monkeys. An array of up to ten stimulating microelectrodes was implanted into superficial layers of the 'arm area' of the motor cortex as identified by muscle contraction to minimal intracortical microstimulation. Single microstimulating pulses antidromically activated neurons of area 5 in the depths of the intraparietal sulcus. Only the anterior bank was explored. Our sample includes only those neurons where a liberal estimate of the spread of threshold current ruled out white matter activation. The relation between effective stimulation sites and the location of antidromically activated neurons was consistent with the topography seen in the HRP studies.

Ten neurons of area 5 projecting to the motor cortex were tested for input from the periphery. Two of these gave weak, phasic responses to abrupt, large amplitude joint movements. Possible contributions of active movements to the responses were not assessed in these alert, untrained animals, which were at times uncooperative. No other responses to peripheral manipulation or spontaneous movements were observed.

The direct connection from area 5 to the motor cortex described in this study could be an important route by which area 5 influences motor cortex output and limb movements. (Supported by the Medical Research Service of the Veterans Administration and by NIH grant 10705).

894 A NEW HYPOTHESIS ON THE FUNCTIONAL ROLE OF MUSCLE RECEPTORS IN SEGMENTAL MOTOR CONTROL. D. G. Stuart and M. D. Binder. Dept. of Physiology, University of Arizona, College of Medicine, Tucson, Arizona, U.S.A.

A relatively small, but influential body of data is now available on the discharge patterns of muscle receptor afferents from cat hindlimb muscles during controlled and unrestrained locomotion. Analogous data exist from a variety of other animal and human muscles during their participation in natural movements. Collating these results, together with those depicting the responses of spindles and tendon organs to single muscle unit contractions and with those delineating the central connections of their afferent fibers, illustrates that traditional models of the functional role of muscle receptors are inadequate and in need of revision. This report presents a new hypothesis on the role of muscle receptors in segmental, spinal motor control mechanisms, together with the evidence on which it is based and the experimental approaches required to test it. We propose that fundamental to the role played by muscle spindles and tendon organs in motor control are their individual capabilities, at the peripheral level, to monitor the activity of a select number of muscle units within the parent muscle and, at the central level, to provide stronger synaptic input to motoneurons innervating the muscle units to which they are responsive than to other motoneurons within the homonymous and functionally related pools. A key corollary to the peripheral component of this hypothesis suggests that rather than acting as generalized, whole muscle length (spindles) and tension (tendon organs) sensors, each individual receptor monitors the activity of a discrete set of muscle units within the parent muscle. The resultant afferent signals are modulated by the overall length-tension status of the muscle and, in the case of spindles, by fusimotor input as well. A key corollary at the central level suggests that all three muscle afferent types (Ia, Ib and spindle group II) contribute to motoneuronal post-discharge inhibition. The Ib afferents do so through polysynaptic inhibition and the spindle afferents through "relative disfacilitation" (i.e., transient reduction in excitatory input to a motoneuron following its muscle unit's contraction). Our presentation will also address the functional distinctions between the information content of single afferent channels and that of the collective input from all receptor afferents from a given muscle. (Supported in part by the Fan Kane Foundation and USPHS Grant NS 07888.)

895 EFFECTS OF ACUTE CORTICAL INJURY ON THE ACTIVATION OF THE MOTOR CORTEX IN THE CAT. Floyd J. Thompson. Dept. Neurosci., Col. Med., Univ. Fla., Gainesville, FL.

The effects of acute cortical injury on the general pattern of sensory transmission to and activation of motor cortex neurones were investigated in the following experiments. Two fundamental questions were examined: are changes in the pattern of cortical neurone activation related only to the degree of proximity to the lesion or are changes in the pattern of cortical activation also related functionally to the cortical region injured? The motor corteces of cats were exposed and covered with a closed chamber. Bipolar concentric electrodes were placed stereotaxically into the contralateral dentate nucleus and in the ipsilateral medullary pyramidal tract. A bipolar concentric electrode was also placed in the posterior intermediate sulcus in the upper cervical spinal cord to activate the contralateral gracile and cuneate tracts. These electrode placements provided cortical evoked potentials elicited by three functionally different but powerful activators of the motor cortex neurones. Effects of acute cortical injury on cortical activation were determined by analyses of the averaged cortical evoked potentials recorded from several cortical positions before and following the production of a focal (radio frequency) lesion at one of the recording sites. These experiments have shown that the cortical evoked potentials (P_1-N_1 amplitudes) recorded from cortical positions proximal to postcruciate lesion sites are significantly larger following the lesion. However, cortical evoked potentials recorded from a more remote medial precruciate region are decreased in amplitude, particularly in the cortical evoked potential elicited by dentate nucleus stimulation. On the other hand, cortical evoked potentials recorded from both proximal (precruciate) and remote (postcruciate) recording sites are significantly larger following focal lesion at the medial precruciate position. These experiments indicate that following focal cortical lesion, the pattern of cortical neurone activation is altered in both the proximal and more remote cortical regions. The nature of the alteration is functionally related to the region of cortex injured and not simply to proximity to the lesion site.

896 THE FORCES PRODUCED BY HINDLIMB MUSCLES IN FREELY MOVING CATS. B. Walmsley*, J. A. Hodgson* and R. E. Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

Forces produced by individual muscles [medial gastrocnemius (MG) and soleus (SOL)] were measured in the respective tendons of insertion using chronically implanted devices. The device consists of a semiconductor strain gauge bonded along one side of a 1 mm thick stainless steel form (Fig. 1; 8-14 mm long, 5-7 mm wide) which has two slots to permit placement on the intact tendon (Fig. 1, stripes) and insulated with Parylene C and Silastic rubber which prevents the gauge from slipping off the tendon. Its output was led to a connector on the cat's back. Gauges that respond linearly to forces up to 10 kg have been used. Muscle electrical activity (EMG) and force data were recorded on FM tape synchronized with videotape records of movements. The results demonstrate that SOL produces virtually the same peak force (1.5-2.0 kg) over the entire range of hindlimb action (from quadripedal standing to 1.2 m vertical jumps) and obviously plays a major role in locomotion (Fig. 2). In contrast, the peak forces from MG vary more widely over the same range of movements, from <500 gm in standing to 10 kg in jumps. Peak MG force was clearly graded with speed of walking (Fig. 2) while that from SOL was not. This difference in the range of movements over which peak MG and SOL forces are graded is very likely related to the differences in the motor unit populations making up these two muscles.

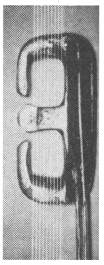


Fig. 1 Photograph of strain gauge device.

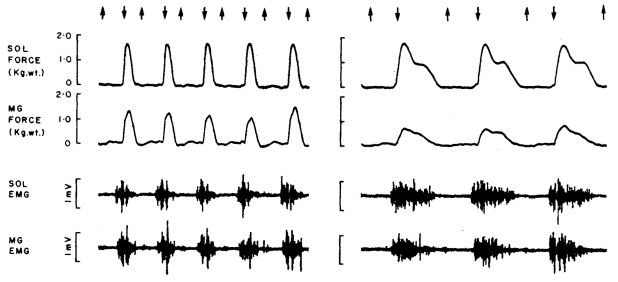


Fig. 2. Fast (left; 1.6 m/sec) and slow (right; 0.8 m/sec) walks in a 2.7 kg cat. ↑ = foot lift; ↓ = foot contact; duration of left record is 2 sec.

897 LINEAR HEAD MOVEMENTS OF WALKING AND TROTTING CATS. Douglas G. D. Watt and Mary C. Wetzel. Aviation Med. Res. Unit, McGill Univ., Montreal, Quebec, Canada H3G 1Y6 and Dept. Psych., Univ. of Ariz., Tucson, Ariz. 85721

Previous work in the cat has demonstrated that natural stimulation of the vestibular otolith organs leads to changes in lumbosacral spinal cord excitability. It has been suggested that vestibulo-spinal reflexes resulting from the cyclical linear accelerations experienced during normal locomotion might contribute to motor control. However, the relationships between head and limb movement have not been examined systematically.

The purpose of this study was to measure the linear head movements of walking and trotting cats, and to relate these to the simultaneously occurring footfall patterns. A light-weight 3-axis linear accelerometer package was mounted on the heads of 6 cats trained previously to walk and trot on an enclosed treadmill. Limb and shoulder movements were measured from high speed movies filmed at the same time.

Stable walking gaits were recorded on 33 occasions, with treadmill speeds ranging from 0.4 to 1.6 m/sec. Trotting was seen 12 times, from 1.2 to 2.7 m/sec. Stride frequency increased linearly with treadmill speed, being 1.28 strides/sec at 0.4 m/sec, and 3.03 strides/sec at 2.7 m/sec. Lateral head accelerations were generally minimal, but those in the fore-aft and vertical directions were substantial and highly reproducible, increasing in amplitude as the rate of progression of the cat increased. A detailed computer-based analysis of head and shoulder vertical movements determined that most of these occurred at twice the stride frequency. The relationship between head and hindlimb movement was not constant within or between cats. However, head movement was closely tied to shoulder, and hence forelimb, movement while the cats walked or trotted.

Otolith-spinal responses could contribute, therefore, to the control of neck and forelimb muscles during locomotion, but would be less likely to participate directly in hindlimb activity. This conclusion is compatible with the finding of Wilson (Int. Rev. of Neurobiol. 15: 27, 1972) that monosynaptic vestibulospinal projections to motoneurons are largely limited to the cervical and upper thoracic spinal cord.

(Supported by USPHS Grant NS-11491)

898 THE AVIAN RUBROSPINAL TRACT: ITS CELLS OF ORIGIN, COURSE AND SITES OF TERMINATION. J. Martin Wild*, John B. Cabot, David H. Cohen, and Harvey J. Karten. Dept. of Physiol., Sch. Med., Univ. of Virginia, Charlottesville, VA 22901 and Dept. Psychiat., Sch. Med., SUNY, Stony Brook, NY 11794.

As part of a series of anatomical studies of the major descending projections to the spinal cord of the pigeon (Columba livia) we have described the cells of origin, course and sites of termination of the rubrospinal tract (RST).

In Nissl-stained material the nucleus ruber of the pigeon cytoarchitectonically resembles the mammalian nucleus ruber, consisting of both magnocellular and parvocellular divisions. The large neurons (40-50 μ) defining the magnocellular portion of the nucleus are located dorsomedially and ventrolaterally at the more caudal nuclear levels, while the small and medium sized neurons (15-35 μ) comprising the parvocellular portion are more prominent at rostral levels. It should be emphasized, however, that neurons of all sizes are present throughout the entire rostrocaudal extent of the nucleus, and at intermediate levels it can be difficult to distinguish magnocellular and parvocellular divisions.

The course and sites of termination of the RST were investigated using either anterograde degeneration or autoradiographic techniques. From such material the axons of rubral neurons could be traced across the midline where they pass the ventrocaudal aspect of the contralateral nucleus ruber and then form a compact bundle which sweeps laterally and rostroventral to the nucleus tegmenti pontinus. The RST continues ventral to and distinctly separate from the brachium conjunctivum and passes ventral to the entering radix of the trigeminal nerve. At rhombencephalic levels the RST lies on the ventrolateral aspect of the brainstem and, more caudally, is situated dorsomedial to the ascending fibers of the dorsal spinocerebellar tract. The RST then enters the spinal cord as a well-defined bundle in the dorsal portion of the lateral funiculus, and its terminations within the spinal gray are limited to the base of dorsal horn in laminae V and VI of Leonard and Cohen (J. Comp. Neurol., 1975, 163:149-180.)

A possible topographic organization of this projection was then investigated by means of the retrograde transport of horseradish peroxidase following injections into the lateral funiculus at brachial, thoracic or lumbar levels of the spinal cord. Regardless of injection level, labelled neurons were prominent throughout the rostrocaudal extent of the contralateral nucleus ruber in both its magnocellular and parvocellular divisions. Thus, if a topographic projection does exist, it is a subtle one with a substantial degree of overlap.

(Supported by NSF grant BNS75-20537 to D.H. Cohen, NIH grant NS-12078 to H.J. Karten, and a grant from the Alfred P. Sloan Foundation to the University of Virginia Neuroscience Program.)

899 ELECTROMAGNETIC STRETCH OF INDIVIDUAL MUSCLES IN BEHAVING PRIMATES. Jonathan R. Wolpaw* and Theodore R. Colburn* (SPON: H. C. Lansdell). Laboratory of Neurophysiology and Section on Technical Development, NIMH, Bethesda, MD 20014.

This report describes a technique for stretch of individual muscles in awake behaving primates by direct application of calibrated force unaccompanied by other stimuli. An external electromagnet applied force to a small piece of permeable metal permanently imbedded in the musculotendinous junction.

A 3 x 5 x 18 mm coated steel implant was sutured inside flexor carpi ulnaris of Macaca mulatta 30% of the way from distal to proximal insertion with its long axis parallel to that of the muscle. A coil was wound on a longitudinally split hollow aluminum spool. Current was supplied either by an operational power supply or by an AC transformer. The monkey sat in a primate chair, elbow flexed to 90^0, and forearm passing through the spool so that the implant was half inside the proximal end of the coil and as near to its longitudinal axis as possible. The major component of the force applied to the implant by the coil was thus directed distally parallel to the long axis of the muscle. Its maximum value was 90 gm for the operational power supply and 158 gm for the AC transformer. The monkey grasped a movable handle and received a periodic liquid reward for maintaining the handle in the mid-position, wrist neither flexed nor extended. Steady state torque, tending to move the handle out of the mid-position, could be applied to the handle via a torque motor. Flexor carpi ulnaris EMG activity was recorded via intra-muscular wire electrodes.

Activation of the coil appeared to cause no distress to the animal nor did it produce any response other than that accompanying elicitation of a stretch reflex. Tendon jerks of 10-14 msec latency followed the onset of 100 msec (7 msec rise time), 90 gm, DC stretch delivered at pseudo-random 3-6 sec intervals. Their amplitude was greater when the muscle was active, opposing external extensor torque on the handle, than when it was inactive. Both an initial stretch reflex and a subsequent tonic vibratory response occurred in response to 3.6 sec, 77 gm, 60 Hz full wave rectified stretch.

The range of possible applications of this technique to research at peripheral, segmental, and suprasegmental levels depends on theoretical and practical considerations concerning the design of the electromagnet, current supply and control, and implant form and magnetic properties.

900 OPERANT CONDITIONING OF MONKEYS FOR TONIC CONTROL FIRING PATTERNS OF NEURONS IN MOTOR CORTEX. Allen R. Wyler. Dept. Neurological Surgery, Univ. of Wash., Seattle, Wash., 98195.

A paradigm has been developed which allows monkeys to be trained with operant conditioning to operantly control the firing patterns (rather than rate) of cortical pyramidal tract neurons. This requires the monkey to change phasic firing patterns to tonic firing patterns for reward. This paradigm also allows quantification of operant behavior such that operant control may be compared between neurons, as well as between monkeys. Factors which might predict which neurons were most easily conditioned were phasic firing patterns, and those neurons involved in movement of distal arm muscles. Factors not predictive were initial firing rate or variance. The application of this paradigm to studying central nervous system pathology, i.e. epilepsy, is discussed.

NARCOTICS AND DRUGS OF ABUSE

901 EFFECTS OF CHRONIC THALAMIC, LIMBIC, AND CORTICAL LESIONS ON NARCOTIC DEPENDENCE AND ABSTINENCE IN THE RAT. M.W. Adler, P.B. Beeton*, E.B. Geller*, and P.L. Gildenberg. Temple Univ. Sch. of Medicine, Phila., Pa. 19140.

Although considerable effort has been directed towards studying areas of the brain involved in the analgesic actions of narcotics, relatively little attention has been focused on elucidating the neuronal pathways mediating narcotic dependence and abstinence. One effective means of investigating the different anatomical pathways that subserve the variety of signs which characterize abstinence is the use of brain lesions. Previous studies have generally been restricted to 1 or 2 areas of the brain, a design which tends to preclude the type of comparative analysis of data necessary to study so complex a picture as narcotic dependence and abstinence. The present experiment is an attempt to clarify the interrelationships between a number of brain structures, the production of dependence, and the abstinence syndrome.

Male Sprague-Dawley rats (270-310g) received either bilateral anodal d.c. lesions (subcortical), tissue aspiration (cortical), or sham operation under pentobarbital anesthesia. Animals were then housed in groups of 4 or 5, and a minimum of 3 months elapsed before narcotic administration was begun. This period was allowed in order to obviate any changes caused by non-specific factors such as edema, anesthesia, altered blood-brain barrier, and altered food and water intake resulting from surgery. Animals were implanted with 2 pellets of M (75 mg. M base each) under light ether anesthesia. After 72 hr, naloxone (N) was injected i.p. (1.0 mg/kg) and the abstinence syndrome assessed on a blind basis. Each brain was removed, and lesion locations verified using luxol blue, cresylecht violet stained serial sections. Histology was the determining factor in the decision to include an animal in a group.

Lesion groups were centre median, parafascicularis, other thalamic areas (primarily ventral and dorsomedial), amygdala (primarily cortico-amygdaloid), lateral septum, hippocampus, posterior cortex (primarily area 17 and parts of 18 and 18a), and frontal cortex (primarily area 10). Signs of abstinence scored included weight loss, jumping, teeth chattering, wet-dog shakes, writhing, chromodacryorrhea, diarrhea, ptosis, flat posture, salivation, and rhinorrhea. Groups were compared with appropriate controls and with each other. Differences in frequency of occurrence of each of the abstinence signs varied little and weight loss did not show any significant differences between groups.

We conclude that once recovery from any acute effects of brain damage is complete, lesions in thalamic, limbic, and cortical areas do not markedly alter M dependence or the N-precipitated abstinence syndrome. (Supported by DA 00049 & DA 00376).

902 $H_3{}^{32}PO_4$ AND (^{3}H)-AMINO ACIDS INCORPORATION DURING MORPHINE WITHDRAWAL. Alemán, V., Camacho, J.L.* Centro de Investigación y de Estudios Avanzados del I.P.N., México 14, D.F. MEXICO.

We were interested to know if protein phosphorylation activity is modified in the nervous tissue during morphine abstinence in morphine dependent rats. If it is modified we wanted to know if this change in protein phosphorylation activity may produce a change in the metabolism of some proteins of the cell.

Groups of six female rats, 25 days old were made morphine addicted by twice dayly injections of increasing doses of morphine for six days.

Fifty µCi of ^{3}H-amino acids mixture was administered intraperitoneally 10, 6 and 2 days before the last morphine injection. In a similar fashion 350 µC of $H_3{}^{32}PO_4$ was given 6 and 2 days before the last morphine injection.

Groups of rats were killed at different times after the last morphine injection and the radiactive precursors incorporated, were quantified.

The morphine treated rats showed a significantly higher incorporation of both radioactive precursors as compared with control animals. Both the nuclear and the mitochondrial fractions showed higher specific activities than other cell fractions.

On time, kinetics of both $H_3{}^{32}PO_4$ and (^{3}H)-amino acid uptake were similar. For most of the cell fractions incorporation was maximum at 24 hours; a second maxima was observed on the fourth day.

Radioactive phospho-L-serine was isolated from protein hydrolyzates and the amount of labelled phosphate was determined. In the morphine treated animals the amount of labeled phospho-L-serine was higher, indicating that protein has been phosphorylated.

903 CHANGES IN THE DURATION OF THE ELECTROENCEPHALOGRAPHIC RESPONSE TO COCAINE DURING CHRONIC ADMINISTRATION OF THE DRUG. H. L. Altshuler, N. R. Burch*, M. Hubler*, and R. Dossett*. Texas Research Institute of Mental Sciences and Baylor College of Medicine, Texas Medical Center, Houston, TX 77030.

The electroencephalographic (EEG) response to intravenous challenge doses of cocaine was measured in rhesus monkeys during a study in which the animals were treated chronically with the drug for two years or longer. The animal subjects were 4-6 kg male rhesus monkeys, which had been implanted with cortical electrodes. The responses were measured during experiments in which pre and post dose spontaneous and photically driven EEG were recorded and the resulting EEG analyzed with period analysis. Previous work from our laboratory has shown that the responses to intravenous challenge doses of cocaine change during chronic drug administration such that the first part of the biphasic EEG response becomes potentiated and the second part of the biphasic response becomes attenuated after long term cocaine therapy. Detailed analysis of the first part of the biphasic response revealed that the response became greater in magnitude and also showed increases in response duration following the intravenous cocaine dose. These changes were most pronounced in the frontal and temporal cortex. Detailed analysis of the increase in duration of the first part of the biphasic response to cocaine provides support for the hypothesis that certain responses to cocaine become greater during chronic drug administration; that is, develop reverse tolerance. The second part of the biphasic response decreases both in magnitude and duration after chronic administration, especially in the temporal and occipital cortex. The observation of reduction in magnitude and duration of that part of the response provides support for the hypothesis that certain aspects of the response to cocaine develop tolerance during chronic administration.

904 EFFECT OF ETHYL ALCOHOL ON BRAIN CYCLIC GMP. William E. Askew and K. D. Charalampous. Department of Psychiatry, Baylor College of Medicine, Houston, Texas 77030.

Cyclic GMP has been implicated as a cholinergic mediator in discrete areas of the mouse brain and has also been identified as specifically involved in GABA-nergic transmission in the cerebellum. Ethanol presumably acts in the central nervous system via major mediators. The effect of acute and chronic ethanol on murine c-GMP, such an mediator, is now presented. Acute administration of ethanol decreased cerebellar C-GMP in a dose-related manner but did not affect pons-medulla C-GMP. Chronic administration of ethanol via the pyrazole inhalation model resulted in a decrease in cerebellar C-GMP at 24 hours which returned to normal levels at 48 and 72 hours. Cyclic GMP levels in the cerebral cortex, pons-medulla, and forebrain were not affected by chronic treatment. The decrease in cerebellar C-GMP was partially due to increased phosphodiesterase activity; however, other factors apparently contributed indirectly to the decrease.

905 ENDORPHIN LEVELS IN RAT PITUITARY. Eugene R. Baizman*, Osman Hassan Osman*, and Brian M. Cox*. (Spon.: Avram Goldstein) Addiction Research Foundation, Palo Alto, CA 94304.

Extending our original studies of endorphin from bovine pituitary gland (Brain Res. 124:523, 1977) to the rat, we have applied a similar glacial acetic acid:acetone extraction procedure to freshly microdissected single lobes of rat pars intermedia, pars nervosa and pars distalis. The extracted material was assayed for inhibition of stereospecific binding of ^{3}H-Etorphine in the standard opiate receptor binding assay. Distribution studies generally confirmed earlier results in bovine glands; pars intermedia has approximately 17-fold greater endorphin content than pars nervosa. Females exhibit a 2-fold higher total endorphin content in the anterior lobe than males (14 ± 2 ID50 units/gland-females, vs 6.5 ± 0.7-males; $p<0.01$, reflecting the larger gland weight. No significant differences were observed between neurointermediate lobes. Total endorphin content in pars distalis from male rats appears maximal at about 10 weeks of age, with a decline at 25 weeks, despite an increase in gland weight. Neurointermediate lobe endorphin content increased significantly from 5 to 10 weeks of age, a trend which continued to 25 weeks, and paralleled the increase in gland weight. Whole pituitaries of neonatal rats were also examined for endorphin content. Administration of 2% NaCl in drinking water, electrolytic lesioning of several hypothalamic regions and continuous 30 or 60 min footshock were among attempts to alter pituitary endorphin levels. 12 h, 48 h, and one week following initiation of NaCl drinking, endorphin activity in both anterior and neurointermediate lobe shows a substantial reduction over controls. These experiments, together with footshock and hypothalamic lesioning will be discussed in terms of chronic, stressful stimuli as potential activators of the pituitary endorphin system. (Supported by Natl. Inst. on Drug Abuse grant DA1199).

906 ARE ENDOGENOUS OPIATES INVOLVED IN THE ANALGESIC ACTION OF NITROUS OXIDE? Barry Berkowitz*, A. Donald Finck* and S. H. Ngai* (Spon: C. Neurath). Roche Inst. Molec. Biol., Nutley,N. J. 07110 and Depts. of Anesthes. and Pharmacol. Columbia Univ. College of Phys. and Surg., New York, New York 10032

In an initial study of nitrous oxide analgesia, we utilized the phenylquinone writhing test in mice and observed some similarities between the effects of nitrous oxide and morphine (Science 194: 967-968, 1976). The objective of this study was to further characterize the nature of nitrous oxide analgesia using other tests and to establish if tolerance to nitrous oxide occurs. In mice, nitrous oxide was analgesic in the acetic acid writhing test. Aspirin and very high doses of alcohol were also active in these tests, however, only nitrous oxide induced analgesia was antagonized by narcotic antagonists. These data indicate the mechanism of action of nitrous oxide analgesia differs from that of the other two drugs. A similar phenomenon of analgesia and antagonism by naloxone occurred in rats. Nitrous oxide produced a dose-related analgesic response in rats (ED50, 67%) as measured by the tail-flick method. Naloxone, 5-30 mg/kg, antagonized nitrous oxide analgesia in rats. A 1 mg/kg dose of the antagonist was not effective. It should be emphasized that when naloxone is used alone, it never produced any hyperalgesia in any of the analgesic tests.

Tolerance developed to the analgesic action of nitrous oxide in both rats and mice. Animals exposed to 75% nitrous oxide for 18-24 hours had a reduced analgesic response to subsequent exposures of the gas. These data show that nitrous oxide is analgesic in a variety of tests and lend support to the hypothesis that nitrous oxide and opiates have a significant pharmacologic resemblance. Ultimately, these drugs may produce similar molecular events in the brain lending to relief of pain. A possibility which we are actively considering is that there is an interaction between nitrous oxide and the opiate receptor-endorphin system.

907 REGIONAL DISTRIBUTION OF β-ENDORPHIN AND ENKEPHALIN IN RAT BRAIN: A BIOCHEMICAL AND CYTOCHEMICAL STUDY. F. Bloom, J. Rossier, E. Battenberg, T. Vargo*, S. Minick*, N. Ling* and R. Guillemin. The Salk Inst., La Jolla, CA 92037

Radioimmunoassays (RIA) for β-endorphin (β-end) and Leu^5-enkephalin (Leu-enk) were developed. β-end RIA shows negligible cross-reaction with α-end, Met-, and Leu-enk. Leu-enk RIA shows a 3.7% cross-reaction with Met-enk and the standard curves for Met- and Leu-enk were completely parallel, therefore this RIA cannot discriminate between Leu- and Met-enk. Values given below (Table I) assume that all enkephalin immunoreactivity was due to Leu-enk. If the rat brain contains no Leu-enk but only Met-enk, the Leu-enk values will reflect cross reactivity, and correct Met-enk concentrations will be obtained by multiplying the Leu-enk by 27.

Table I: Regional distribution of β-end and Leu-enk

Tissue	β-end, ng/gm	Leu-enk, ng/gm wet tissue	
Pituitary	269,000. ± 20,000.	75. ± 5.	n = 11
Hypothalamus	490. ± 30.	119. ± 6.	n = 5
Septum	234. ± 34.	85. ± 7.	n = 5
Midbrain	207. ± 15.	29. ± 2.	n = 5
Striatum	< 200	64. ± 9.	n = 5
Medulla + Pons	< 200	19. ± 3.	n = 5

For immunocytology, brains (rats, mice) were fixed by perfusion with 5% p-formaldehyde. Sections were cut on a cryostat and prepared with the PAP method of Sternberger using antisera (AS) to β-end or to enk. With either AS discrete nerve fibers and terminal boutons were observed throughout the CNS. With β-end AS, innervations was most intense in the ventral septum, the preoptic area of the hypothalamus, the paraventricular hypothalamus, the peri-aqueductal grey regions of the thalamus and midbrain and was also observed within portions of the substantia nigra and locus coeruleus. Globus pallidus and caudate nuclei, which show intense staining for enk, do not stain with β-end AS. With the β-end AS, labelled synaptic terminals have been seen by electron microscopy. Neuronal perikarya stained with β-end AS have been seen in the ventral hypothalamus and in the amygdala. These results as well as other endocrine and pharmacological experiments in progress suggest that endorphin-containing neuronal systems exist separately from those containing enkephalin.

908 OPIATES REGULATE ADENYLATE CYCLASE AND PROTEIN SYNTHESIS IN RAT FOREBRAIN REGIONS. K.A. Bonnet and S. Gusik.* New York University Medical Center, New York 10016.

Opiate analgesics and opioid peptides act through specific receptors to effect changes in cyclic nucleotide regulation in brain. In vivo, initial morphine injection results in lowering of midbrain cAMP and cGMP levels and an elevation of cAMP levels in the caudate and thalamus(Life Sciences, 16: 1877, 1975). In vitro, morphine and enkephalin stimulate caudate-thalamus adenylate cyclase activity only in the presence of Ca^{2+}. Morphine microinjected into the thalamus results in substantial analgesia with tolerance development on repeated microinjection. Systemic injection of theophylline (50mg/Kg) elevates cerebral cAMP and cGMP levels somewhat, and significantly potentiates morphine analgesia only if given 30 minutes prior to morphine. Ro20-1724 injected systemically significantly elevates cerebral cAMP only, and produces mild analgesia; injected 30 minutes prior to morphine the result is significantly greater than additive analgesia. By in vivo labelling studies using a double isotope technique we find significant alterations in the populations of high molecular weight RNA in forebrain regions 2.5 hours after the initial encounter with morphine, but not in the pons-medulla or in the cerebellum. These alterations are not the result of changes in RNA synthesis rates. None of these alterations in RNA populations are evident on challenge morphine injection in morphine tolerant (72 hour morphine pelleted) animals. These data provide biochemical evidence for the role of protein synthesis in the development of tolerance to narcotic analgesics. Such alterations appear to be qualitative rather than quantitative, and appear to result from opiate-receptor mediated alterations in cAMP levels since morphine in combination with either phosphodiesterase inhibitor resulted in accelerated tolerance development.

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909 EFFECTS OF CHOLINE ON MORPHINE ANALGESIA IN THE RAT. L. J. Botticelli, L. D. Lytle, and R. J. Wurtman. Massachusetts Institute of Technology, Cambridge, MA 02139.

The administration of choline has been shown to increase levels of acetylcholine in peripheral tissues and brain. Indirect pharmacological evidence suggests that these increases may be associated with a change in the amount of neurotransmitter actually released. Considerable evidence suggests that some actions of morphine and its congeners may be mediated in part by acetylcholine. We therefore studied the extent to which choline, as a precursor of acetylcholine, might modify the response to morphine in the rat. Latency in response to a thermal stimulus was used as the criterion for analgesia. The time required for characteristic lifting of a hindlimb was measured on a modified hot plate device. Difference between latency of the withdrawal response and the altered latency after morphine administration was used as a measure of the analgesic effect. Groups of 10-12 adult male rats received choline chloride (15, 30, or 60 mg/kg, i.p.) and, after 30 minutes, morphine sulfate (10 mg/kg, s.c.). Analgesia was examined 15, 30, 60, and 120 minutes after morphine injection. To evaluate the antinociceptive response, the area under each curve relating drug dose to time and response latency was calculated. Data are given as this area, in minute-seconds, ± S.E.M.

Drug		Analgesic area
Choline Cl (mg/kg)	Morphine SO_4 (mg/kg)	(minute-seconds)
0	0	12 ± 16*
60	0	66 ± 24*
0	10	1285 ± 81
15	10	1022 ± 98
30	10	693 ± 40*
60	10	243 ± 47*

*P < 0.001 differs from group receiving morphine alone.

Results suggest that choline, by increasing acetylcholine release at cholinergic synapses, produces a dose-related antagonism of the analgesic effects of morphine. (Supported in part by U. S. Public Health Service Grant MH-28783.)

910 Δ^9-TETRAHYDROCANNABINOL BLOCK OF 5-HYDROXYTRYPTAMINE STIMULATED RELEASE OF PROLACTIN IN MALE RATS. Bruce L. Bromley, J. H. Gordon and E. Zimmermann, Department of Anatomy and Brain Research Institute, University of California, Los Angeles, CA 90024.

The acute administration of Δ^9-tetrahydrocannabinol (Δ^9-THC) lowers resting levels and blocks the ether-induced rise in plasma prolactin (PRL) in male rats (Bromley et al, Fed. Proc. 36: 1026, 1977). To further study the mechanism(s) by which Δ^9-THC inhibits PRL release, adult male rats received ip injections of 5 or 30 mg/kg Δ^9-THC (2 hrs prior to sacrifice) and 1 hr later they received either a dopamine (DA) receptor blocker (pimozide, 2.5 mg/kg, haloperidol, 1 mg/kg, or chlorpromazine, 25 mg/kg) or a 5-Hydroxytryptamine (5-HT) agonist (fenfluramine, 5 mg/kg, or quipazine, 10 mg/kg). In a second series of experiments male rats received either reserpine (5 mg/kg, 5 hrs prior to sacrifice) or α-methyltyrosine (250 mg/kg 3 hrs prior to sacrifice) followed by Δ^9-THC at 2 hrs prior to sacrifice. All drug doses were selected on the basis of either preliminary studies or published values which produced a significant elevation in plasma PRL. All animals were decapitated, within 30 sec following cage opening, and trunk blood was collected for radioimmunoassay of PRL. In the absence of Δ^9-THC treatment each of the drugs caused a significant ($p<0.05$) elevation of plasma PRL levels. Δ^9-THC treatment failed to alter the prolactin response to pimozide and chlorpromazine, but partially reduced the response to haloperidol. In contrast, Δ^9-THC completely abolished the PRL response to fenfluramine (which releases central stores of 5-HT) and quipazine (which directly stimulates 5-HT receptors). Δ^9-THC treatment did not antagonize the PRL elevating effects of α-methyltyrosine and only partially antagonized the effects of reserpine.

Both DA and 5-HT have been implicated in the regulation of PRL release. DA has been proposed to act as a PRL release inhibiting factor (PIF) at the level of the pituitary; conversely, 5-HT has been proposed to stimulate the release of a PRL releasing factor (PRF). When either the level of DA is reduced (reserpine and α-methyltyrosine) or its effectiveness at receptor sites is reduced (pimozide, haloperidol and chlorpromazine) then the Δ^9-THC treatment at best only partially inhibited the drug induced rise in plasma PRL. Conversely, when 5-HT agonists were used to stimulate a PRL release, then Δ^9-THC was a potent inhibitor of PRL release. These data suggest that Δ^9-THC either inhibits the action of 5-HT or the release and/or action of PRF. (Supported by USPHS grants DA826 and DA5010).

911 BEHAVIORAL CHARACTERIZATION OF THE EFFECTS OF ß-ENDORPHIN, NEUROLEPTICS AND OPIATES. Ronald G. Browne and David S. Segal. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA 92093.

Recently Bloom et al. (Science 194: 630, 1976) reported that intraventricular administration of ß-endorphin in rats produces a profound state of immobilization characterized by the absence of movement, loss of righting response and extreme generalized muscular rigidity. However, Jacquet and Marks (Science 194: 632, 1976) reported that injections of ß-endorphin into the periaqueductal gray (PAG) elicit a "cataleptic-like" state similar to that produced by most neuroleptics. Differentiation of the effects of ß-endorphin as rigid immobility or neuroleptic-like catalepsy may be crucial, both with respect to mechanistic considerations as well as possible clinical implications. Therefore, we have extended our earlier studies to characterize more completely the state of immobility produced by ß-endorphin and to compare it with the behavioral profile produced by opiates and neuroleptics. Following the systemic, intraventricular or PAG administration of these drugs, rats were tested for: rigidity, immobility on a vertical grid, righting reflex and gross behavioral and physiological changes. A dose-response analysis of intraventricular or PAG administered ß-endorphin revealed that this neuropeptide induced a state of immobility characterized by general muscular rigidity, loss of righting reflex and negative response on the vertical grid test. In contrast, haloperidol (0.5 - 12 mg/kg, s.c.) treated rats did not exhibit rigidity, maintained their righting reflex and remained stationary for at least 60 sec on a vertical grid. Pharmacological interactions further differentiated the effects of these two drugs. All the effects of ß-endorphin, but not those produced by haloperidol, were reversed by naloxone (0.1 - 2 mg/kg, s.c.). Furthermore, animals made rigid with ß-endorphin and later injected with haloperidol (2 mg/kg) became flaccid. In rats rendered flaccid by this combined treatment, naloxone administration resulted in the emergence of the typical spectrum of haloperidol effects. These results do not support the contention that ß-endorphin is an endogenous neuroleptic (Jacquet and Marks, 1976). We also found that the synthetic opiate methadone produced a pattern of effects which closely resembles the ß-endorphin-induced syndrome, indicating that the similarity between ß-endorphin and opiates such as methadone extends beyond their analgesic properties, and therefore that they may exert their effects through the same spectrum of neurochemical actions.

912 ETONITAZENE AS A REINFORCER FOR RATS: TASTE, OLFACTORY, AND POSTINGESTIONAL FACTORS. Marilyn E. Carroll* and Richard A. Meisch* (SPON: M.E. Ruggero) Department of Psychiatry, University of Minnesota, Minneapolis, MN 55455.

Previous studies have demonstrated that etonitazene HCl (ETZ) may function as a reinforcer for rats. Since etonitazene (5 or 10 µg/ml) is believed to be a relatively tasteless compound, in most studies the drug has been tagged with a taste additive or paired with visual stimuli. The present study investigated the reinforcing properties of ETZ with and without auditory discriminative stimuli. ETZ and water were concurrently available to 6 rats during daily 1-hr sessions in operant conditioning chambers equipped with 2 levers and 2 liquid dippers. A food-induced acquisition procedure was used whereby ETZ rapidly served as a reinforcer (i.e., is preferred to water) for food-deprived rats. Drug and water positions and liquid containers were alternated on a random basis.

Etonitazene maintained significantly higher response rates than water in the presence and absence of auditory discriminative stimuli even as ETZ concentrations were reduced logarhythmically from 10.00 µg/ml to .078 µg/ml. Drug intake (µg/kg) increased directly with concentration, whereas dipper presentations first increased and then decreased. Even at the lowest concentrations, the rats reliably chose the drug-lever from the onset of the session. These results suggest that drug choice was made on the basis of taste or olfaction rather than postingestional factors. The role of taste vs. olfactory factors were studied by presenting full vs. empty dippers at the start of the sessions. Also, an extinction test was conducted in which dipper operation was prevented but liquid reservoirs were present.

913 ASSAY OF METHIONINE- AND LEUCINE-ENKEPHALIN IN HUMAN BRAIN AND CEREBROSPINAL FLUID. Steven R. Childers* and Solomon H. Snyder. Depts. of Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205

Levels of opioid peptides were measured in human brain to determine whether the enkephalin pentapeptides are the predominate opioid peptides and to determine the ratios of methionine-enkephalin (met-enk; H_2-TYR-GLY-GLY-PHE-MET-OH) to leucine-enkephalin (leu-enk; H_2-TYR-GLY-GLY-PHE-LEU-OH). Adult human brain samples obtained 6-12 hours post-mortem were dissected, extracted in 0.1 N HCl, neutralized and lyophilized. Total opioid peptide levels were determined by rat brain opiate radioreceptor assay using ^{3}H-naloxone as ligand. Levels of met-enk and leu-enk were determined by radioimmunoassays utilizing specific antisera directed against each peptide. Regional dissection studies revealed that highest levels of enkephalin occurred in basal ganglia: globus pallidus > putamen $\geq$ caudate. In the caudate nucleus, enkephalin was concentrated in the head region, with lower amounts in the caudate body and tail. In the basal ganglia, levels of met-enk + leu-enk approximately equaled total opioid peptide levels, indicating that enkephalin is the predominate opioid peptide. Met-enk was higher than leu-enk, with met-enk:leu-enk ratios between 4 and 12. Hypothalamus and periaqueductal gray contained lower enkephalin levels. All cortex and cerebellum areas, along with thalamus, amygdala, medulla, and pons contained negligible opioid peptide levels. Inferior colliculus contained large quantities of opioid peptides by radioreceptor assay, but negligible enkephalin by radioimmunoassay. Therefore, this area may contain significant amounts of non-enkephalin opioid peptide (s).

Radioimmunoassays of human CSF revealed no detectable activity of either met-enk or leu-enk (< 1 pmole/ml). Radioreceptor assay detected only a small amount of opioid peptide activity which eluted from Biogel P-2 columns with higher molecular weight than enkephalin. (Supported by USPHS grants DA-00266 and MH-33128).

914 A COMPARISON OF DIBUTYRYL CYCLIC GMP AND MORPHINE INDUCED ANTINOCICEPTION. Major L. Cohn and Marthe Cohn*, Dept. Anesthesiology, Magee-Womens Hosp., Univ. Pgh. Sch. Med., Pittsburgh, PA 15213.

The analgetic properties of the opiates are not dissociable from systemic and central nervous system depression. We have previously reported the original findings that unlike morphine, the dibutyryl analog of the nucleotide guanosine 3':5' cyclic monophosphate (db cyclic GMP) is a potent analgetic devoid of systemic and central nervous system depression. In the present study, we examined whether db cyclic GMP induces dependence and suppresses the acute morphine abstinence syndrome. Following microinjections in specific sites of the brain, we also compared the analgetic responses and behavioral events produced by db cyclic GMP and morphine. Groups of Sprague-Dawley male rats (200-250 g) were stereotaxically implanted with cannulae either in the lateral ventricle of the brain (ICV), in the periaqueductal gray matter (PAG) or in the mid-brain reticular formation (MRF). Either db cyclic GMP (5-200 µg), morphine sulfate (5-30 µg) or saline vehicle was administered through the cannulae. Analgesia was tested by a gradient increase of temperature on a hot plate. While rats treated with db cyclic GMP (150-200 µg) exhibited no alteration of either behavior or locomotor activity (recorded on Stoelting Activity Monitor), they tolerated temperatures up to 60°C without any signs of discomfort or sedation. Of considerable potential is our finding that all db cyclic GMP treated rats survived temperatures beyond 54°C whereas rats treated with morphine died within 1 to 4 hours after the experiment. Naloxone (1 mg/kg) injected subcutaneously (s.c.) did not alter the analgetic properties of db cyclic GMP. The continuous ICV perfusion of db cyclic GMP for three days (total dose 8 mg/rat) produced no behavioral alteration. Neither the abrupt cessation of the perfusion nor naloxone (1 mg/kg) injected s.c. precipitated withdrawal symptoms. In rats addicted with morphine (150 mg/kg) and challenged with naloxone (1 mg/kg) s.c. db cyclic GMP (200 µg) did not suppress withdrawal symptoms. Microinjections of db cyclic GMP (200 µg) into PAG or MRF did neither suppress noxious stimuli nor produced behavioral events induced in these sites by microinjections of morphine. Our evidence suggests that db cyclic GMP 1) is a potent analgetic devoid of systemic and central nervous system depression; 2) protects against death secondary to heat exposure; 3) does not induce dependence; 4) is not blocked by naloxone; 5) does not produce analgesia at the same sites as morphine; 6) does not induce the same behavioral events as morphine. In conclusion, the mechanism of action of db cyclic GMP seems mediated through a pain inhibitory pathway distinctly different of that upon which morphine acts.

915 ACUTE EFFECT OF ETHANOL ON HIPPOCAMPAL CA1 FIELD POTENTIALS RECORDED IN VITRO. William A. Corrigall, Peter L. Carlen and Allan L. Staiman. Dept. Medicine (Neurology),Addiction Research Foundation, Toronto, Ontario, Canada, M5S 2S1.

The effect of ethanol on field potentials recorded in the CA1 region of the transverse hippocampal slice from the rat has been examined. Potentials were evoked by stimulation in the stratum radiatum (orthodromic) or in the alveus (antidromic). Ethanol was bath-applied over a concentration range of 0-600mM (0-4.6%).

At the highest concentrations (consistently at 600mM), the field potential evoked by orthodromic stimulation was abolished within several minutes. Stimulation of the alveus would, however, still give rise to an antidromic field potential, although reduced in amplitude by 40 to 75% of control. Recordings of activity following stratum radiatum stimulation occasionally included a small negative spike which preceeded the population spike by approximately 1 msec and which, unlike the population spike, did not facilitate following tetanus. This spike may represent activity in the presynaptic fibers. Ethanol at 600mM completely blocked this spike indicating that, if the above assignment is correct, the effects on the orthodromic field potential at this concentration may be due exclusively to conduction block in the afferent fibers.

At 400mM ethanol, the radiatum-evoked field potential was reduced in amplitude by 25-50% and occurred at longer latency. In addition, in those cases in which responses in control medium consisted of several spikes, the longest latency spikes were frequently abolished. The "presynaptic spike" also was reduced at this concentration but to a lesser extent than the post-synaptic potential. The antidromic field potential was reduced by approximately 25%.

At 100mM ethanol, reductions of as much as 25% of the amplitude of the orthodromic field potential could still be observed, although the "presynaptic spike" appeared to have been unaffected at higher concentration (200mM).

All of these effects were reversible upon return to control medium.

Preliminary data at the low concentrations (100-200mM) does not indicate any greater sensitivity to ethanol of post-tetanically or double-pulse facilitated responses, although subtle effects at lower concentrations may be more evident in data obtained intracellularly.

(supported by The Medical Research Council of Canada)

916 ABRUPT WITHDRAWAL OF BUPRENORPHINE, ETHYLKETOCYCLAZOCINE, KETOCYCLAZOCINE OR PENTAZOCINE IN THE RAT: EFFECT ON SEIZURE SUSCEPTIBILITY AND BODY WEIGHT. A. Cowan* and M.W. Adler. (SPON: D.L. Margules). Temple Univ. Sch. of Med., Philadelphia, Pa. 19140.

Whereas acute doses of morphine (M) increase the threshold to flurothyl-induced convulsions in rats, the M primary abstinence syndrome in this species is associated with a decrease in the threshold (Adler *et al.* Psychopharm. *35*:243,1974). How useful is the latter finding in the evaluation of those analgesics that are claimed to possess only low physical dependence capacities? This question seemed particularly pertinent since, in our hands, analgesics of current interest could be differentiated after acute injection i.e. buprenorphine (B) was anticonvulsant, pentazocine (P) was proconvulsant, and ethylketocyclazocine (EK) and ketocyclazocine (K) had no marked effect. We now describe the effect of abrupt withdrawal of these compounds on seizure threshold (S.T.) and on body weight, a commonly reported sign of withdrawal in the rat.

Groups of 8-10 male, albino Sprague-Dawley rats (300-350 g) were injected s.c. at 0800 and 1700 h daily for 10 days with either saline (S), B, EK (both 1.0-16 mg/kg), K (0.50-8.0 mg/kg), P (2.0-32 mg/kg), or morphine (10-160 mg/kg). Each rat was weighed at 0900 and 1700 h daily during the withdrawal period. The flurothyl challenge took place 40 h after the last injection.

Seizure threshold and mean % weight change during withdrawal

	% wt change +40 h; 9 am	S.T. at +40 h mean ± s.e. (sec)	% wt change +64 h; 9 am
S	4.9	332 ± 13	6.4
M	-14.1	276 ± 10*	-14.9
B	2.8	389 ± 14*	1.7
EK	-4.9	336 ± 16	-2.2
K	1.4	295 ± 9	1.9
P	1.7	338 ± 23	1.6

*P<0.05 (Dunnett's method for multiple comparisons)

To summarize our major findings: a) pronounced effects on both S.T. and body weight were only obtained with rats undergoing withdrawal from M; b) in rats treated with B, EK, K, or P and then withdrawn there was no significant decrease in S.T.; indeed, there was a marked increase after B; c) rats treated chronically with EK lost weight during withdrawal.

This pilot study has shown that by recording weight loss and seizure threshold it may be possible to characterize the withdrawal syndromes of analgesics with low physical dependence capacities. (Supported by grant DA 00376 from NIDA. AC is the recipient of a Wellcome Travel Grant).

917 SELECTIVE OPIATE DEPRESSION OF SENSORY-EVOKED SYNAPTIC NETWORKS IN DORSAL-HORN REGIONS OF SPINAL CORD CULTURES. Stanley M. Crain, Edith R. Peterson*, Bea Crain* and Eric J. Simon*. Depts. of Neuroscience and Physiology, Albert Einstein Coll.Med., Bx., N.Y. 10461; Dept. of Medicine, New York Univ. Coll.Med., N.Y. 10016.

Prominent sensory-evoked synaptic networks develop in dorsal horn regions of fetal mouse spinal cord cross-sectional explants, with attached dorsal root ganglia (DRGs), cultured in high NGF (Cr.&Pet., Br.Res.79:145,'74; Soc.Neurosci.'75,'76). Focal DRG stimuli elicited complex negative slow-wave responses restricted to dorsal cord loci, with latencies of 2-3 msec and duration often > 500 msec, resembling primary and secondary sensory-evoked synaptic network responses in situ. These DRG-evoked dorsal cord responses are maintained or augmented in 10^{-3}M GABA, whereas ventral cord discharges are rapidly depressed. Recordings were made via saline-filled pipettes, 3-5 μ tips; electric stimuli (0.5 msec) were applied via 10 μ pipettes.

Morphine sulfate at 10^{-7}-10^{-6}M often led to sustained depression of major components of the DRG-evoked negative slow-wave responses in dorsal cord within 3-10 min, whereas ventral cord discharges were either unaltered or enhanced. Etorphine produced similar selective depression of the dorsal cord responses at still lower concentrations (10^{-8}-10^{-7}M). Levorphanol was comparable in potency to morphine, whereas dextrorphan was ineffective at 10^{-6}M. Introduction of naloxone at 10^{-8}-10^{-6}M restored opiate-blocked cord responses within minutes, whereas recovery in BSS required much longer periods. Naloxone also prevented effects of subsequent addition of morphine or etorphine. In many cultures, naloxone elicited increases in amplitude and duration of the DRG-evoked dorsal cord responses even when introduced without prior opiate exposure.

The concentrations of morphine which depressed sensory-evoked dorsal-cord networks in vitro compare well with analgesic levels in situ, and the antagonist effects of low concentrations of naloxone support the validity of this tissue culture model for studies of opiate mechanisms in the CNS. Localization of opiate sensitivity in dorsal horn regions of cord-DRG explants is consonant with studies in situ and with evidence of high opiate receptor binding levels in these cultures, especially in DRG neurites (Simon et al., this vol.). This study is the first to demonstrate that sensory-evoked synaptic networks in the dorsal horn regions of spinal cord tissue cultures can be selectively depressed by exposure to opiates. Furthermore, naloxone-enhancement of DRG-evoked dorsal cord responses in cultures not exposed to exogenous opiates suggests that these sensory CNS networks may develop localized opioid inhibitory control systems as in situ.

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918 DEGRADATION OF ENKEPHALINS. Gale L. Craviso* and José M. Musacchio. Dept. Pharmacol., Sch. Med., NYU, New York, NY 10016.

We have studied the degradation of enkephalins by the guinea pig myenteric plexus-longitudinal muscle (MPLM) strip in order to isolate the endogenous ligand which is released by the electrical stimulation of the same preparation (Puig et al., Science 195: 419,1977). MPLM strips were incubated with ^{3}H-Met^5-enkephalin or ^{3}H-Leu-enkephalin (2 x 10^{-8} M) in Krebs bicarbonate at 37°. At the end of the incubation, the strips were removed and HCl added. Enkephalin and its degradation products were analyzed by TLC. Almost all the enkephalin degraded could be accounted for as free tyrosine, indicating that the enkephalin pentapeptides were cleaved at the Tyr-Gly bond.

We found that even after extensive washings of the MPLM strips, considerable peptidase activity leaches out of this preparation into the Krebs medium. We studied this leaching enzyme and compared it to the peptidase activity of guinea pig serum and brain subcellular fractions. The peptidases were incubated in Hepes buffer at pH 7 and 37° with ^{3}H-Met-enkephalin (2 x 10^{-8} M). After the addition of HCl, aliquots were analyzed by TLC. The major degradation product obtained by each source of the peptidases was free tyrosine. Boiling at 100° for 2 min destroyed peptidase activity in all cases. As can be observed in the following table, the peptidase activity of the various sources was different as indicated by the percent inhibition produced by certain inhibitors. The concentration of enzyme from the different sources was adjusted so that the control samples degraded 40% of the enkephalin added.

	100,000 x g Brain Sup	MPLM Sup	Serum	MPLM Leaching
Met-Enkephalin 10^{-4} M	73	78	42	51
Bacitracin 100 γ/ml	97	99	21	68
Lima Bean Trypsin Inhib. 100 γ/ml	65	75	4	18
o-Phenanthroline 10^{-3} M	100	100	100	100

From this table, it can be concluded that the peptidases of the 100,000 x g sup from brain and MPLM are similar and that they are different from the serum and MPLM leaching peptidases.

(Supported by USPHS-NIMH grants DA 00351 and MH-17785.)

919 NORADRENERGIC ROLE IN THE SELF-ADMINISTRATION OF ALCOHOL BY RATS. W.M. Davis, S.G. Smith and T.E. Werner. Department of Pharmacology, School of Pharmacy, University of Mississippi, University, MS 38677.

The acquisition by rats of a lever-response reinforced by intragastric (i.g.) infusions of alcohol has been described previously (Davis et al., Proc. West. Pharmacol. Soc. 19: 346, 1976). The role of noradrenergic processes in such self-administration behavior was assessed in rats allowed to lever-press for 25 mg/kg i.g. doses of alcohol. Cannulation for i.g. infusion was by an esophageal approach (Smith et al., Physiol. Psychol. 3: 220, 1975). Access to infusions of saline for establishing an operant baseline was followed by sessions on acquisition contingencies for alcohol or for sweet milk (sweetened condensed milk diluted to half strength). Following acquisition of the lever-press response, the saline contingency was reintroduced, i.e., extinction was imposed. Prior to a reacquisition session, rats were treated with saline, with an agent depleting both norepinephrine (NE) and dopamine (DA), α-methyltyrosine (225 mg/kg), with an agent depleting only NE, 1-phenyl-3-(2-thiazolyl)-2-thiourea (U-14,624; 600 or 300 mg/kg), or with an agent blocking DA receptors, haloperidol (3.5 mg/kg). Results showed that both the control subjects (i.e., the saline-pretreated alcohol group and the sweet milk groups with all three pretreatments) and the haloperidol-treated subjects all reacquired lever-press behavior, whereas the other groups did not. The latter results are attributed to abolition of the reinforcing effect of the infusion of alcohol. The results with the sweet milk reinforcer indicate that the effects on alcohol self-administration of drugs altering central neuroamine functions are not caused by an abolition of all appetitive behaviors and reinforcement. Brain levels of NE, DA and serotonin (5-HT) were measured following administration of the amine-depleting compounds employed in the reacquisition tests. NE was depleted by both compounds, DA was depleted only by α-methyltyrosine, and 5-HT was elevated by 600 mg/kg of U-14,624, but unaffected by 300 mg/kg. As the lower dose of U-14,624 had the same behavioral effect as the higher one, but without elevating 5-HT, it seems unlikely that the behavioral effect involves 5-HT. These results suggest that NE has an important function in the reinforcing action of alcohol, whereas DA does not. (Supported by research grant AA 01217-02 from NIAAA, and in part by the Research Institute of Pharmaceutical Sciences, the University of Mississippi).

920 CORRELATION OF OPIATE LOCALIZATION AND PHARMACOLOGICAL ACTION IN THE MYENTERIC PLEXUS OF THE GUINEA PIG ILEUM. Ihsan M. Diab, Bruce H. Wainer* and Lloyd J. Roth* (SPON: J. de la Torre). Depts. of Pharmacological and Physiological Sciences, Psychiatry, and Pathology. University of Chicago, Chicago, IL 60637.

Morphological localization of three narcotic agonists was determined autoradiographically and compared with their pharmacological effects on the electrically stimulated longitudinal muscle-myenteric plexus preparation.

Concentrations of 100nM ^{3}H-morphine, 0.100nM ^{3}H-etorphine, or 1550nM ^{3}H-met-enkephalin resulted in 90-100% inhibition of the ileal contractile response in each case. These inhibitions were reversed by the presence of 10nM naloxone in the bath or by washing the preparation.

Various experimental manipulations were performed following the addition of each agonist. At the termination of each manipulation the tissue was removed from the organ bath, the longitudinal muscle-myenteric plexus strips were separated from the intact ileal segments and processed for autoradiography.

Autoradiograms of plexus preparations removed from the bath at maximum inhibition by ^{3}H-morphine, without washing, showed high radioactivity over the longitudinal muscle with little activity over the myenteric plexus proper. Occasional satellite cells were found to be labeled. Autoradiograms from strips maximally inhibited by ^{3}H-morphine and reversed by naloxone, without washing, showed a radioactive distribution pattern similar to ^{3}H-morphine autoradiograms described above. Autoradiograms of tissue maximally inhibited by ^{3}H-morphine followed by washing and allowed to recover full contractility, showed silver grain density which was considerably reduced over the longitudinal muscle. Within the plexus proper, occasional satellite cells were radioactively labeled. Autoradiograms from tissue maximally inhibited by ^{3}H-morphine and reversed by naloxone, followed by washing, showed a low distribution of silver grain density on the longitudinal muscle with no radioactivity associated with satellite cells. In no autoradiograms was there any definite neuronal labelling observed in the myenteric plexus. Similar autoradiographic results were obtained with ileal myenteric-plexus preparations treated with either ^{3}H-etorphine or ^{3}H-met-enkephalin. The present studies show no correlation between labelling of a particular cell type and the contractile state of the smooth muscle. Furthermore, there appears to be a selective barrier to the penetration of narcotic agonists into the myenteric plexus of the guinea pig ileum. This barrier may reside in the basal lamina sheath that envelops the plexus, and the narcotic agonist action may be mediated at the surface of the sheath. Supported by grants USPHS RO1 DA00393-05 & RO1 DA00397-05.

921 A COMPARISON OF THREE METHODS FOR PRODUCING PHYSICAL DEPENDENCE ON MORPHINE IN THE RAT. William A. Dickinson, Robert A. Levitt, and Beth L. Eskanazi*. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, Il. 62901

The laboratory rat is a popularly employed subject in the study of narcotic dependence. Three main methods for the production of physical dependence on morphine in rats have evolved to date. These methods can be categorized according to the routes of administration and include parenteral injection, oral ingestion, and pellet implantation. This study compares the effectiveness of these three methods in producing physical dependence on morphine in the rat as measured by the severity of the withdrawal syndrome during naloxone precipitated abstinence.

Three groups of 10 rats were made physically dependent on morphine. Group 1 received twice daily injections (IP) of 25mg. morphine/kg. body weight for a period of 3 days. Group 2 was provided with an ad lib choice between water and a morphine-sucrose solution (0.25mg. morphine/cc in 10% sucrose) for 10 days. Group 3 recieved their morphine for 3 days via a 75mg. morphine-base pellet implanted subcutaneously in the cervical region of the back. Withdrawal was precipitated at the end of the dependence periods by an I.P. injection of 5mg. naloxone hydrochloride/kg. body weight. Six similarly treated groups (N=10) were added as controls. Groups 4, 5, and 6 were administered morphine as in groups 1, 2, and 3 respectively, but were injected with physiologic saline solution rather than naloxone on the withdrawal day. Groups 7, 8, and 9 did not receive morphine at any time, but were injected twice daily for three days (group 7), provided with unadulterated sucrose solution (group 8), and implanted with placebo pellets (group 9). These latter 3 groups were injected on the withdrawal day with 5mg./kg. naloxone as a control for its effects. Immediately following the precipitation of withdrawal all subjects were placed in 1 gal. size "mayonaise type" jars for a period of 1 hr. during which 13 behavioral signs of withdrawal were recorded.

Analysis of the results indicated no significance difference between the severity of the naloxone-precipitated withdrawal syndrome produced by the 3 different methods of administering morphine. There was a significant difference ($p<.001$) between the groups treated with morphine followed by naloxone (groups 1-3) and the control groups (groups 4-9).

It appears, from the results of this study, that the major consideration in choosing which of these three popular methods of producing physical dependence on morphine in the rat are ones of convenience and economy since all three methods consistently produce a relatively severe withdrawal syndrome.

922 STUDIES ON THE GENERALITY OF CALCIUM ENHANCEMENT OF ALCOHOL AND HYPNOTIC DRUG INTOXICATION IN RATS. C.K. Erickson, L.K. Beck*, K.L. Duensing*, and R.M. Huff*. Dept. Pharmacol. Toxicol., Sch. Pharm., Univ. Ks., Lawrence, KS 66045.

Earlier studies (Harris, Fed. Proc. 36: 285, 1977; Tyler and Erickson, ibid. 36: 331, 1977) have shown that calcium chloride (Ca^{2+}) and some other cations will significantly enhance ethanol-induced sleeping-time in mice. Ca^{2+} also enhances sleeping-time produced by tertiary butanol, chloral hydrate, and pentobarbital in mice. To test the generality of the enhancement in another species, and with lower doses of these hypnotics, rats were given the same four CNS depressants intraperitoneally at various times after intracerebroventricularly-administered Ca^{2+}. High (sleep-inducing) doses and low doses which produced motor incoordination were used. Motor coordination was measured by the moving belt ("treadmill") apparatus of Gibbins et al. (J. Pharmacol. Exp. Ther. 159: 236, 1968). The results in the sleeping-time studies were identical to those seen earlier in mice, when a range of Ca^{2+} doses was used, and when the Ca^{2+} doses were adjusted for brain weight. Furthermore, the Ca^{2+} effects on ethanol-induced sleeping-time were increased by pretreatment with Ca^{2+} ionophores (X537A and A23187) in rats as reported earlier in mice. In the treadmill studies, various doses of Ca^{2+} shift the ethanol dose-response curve dramatically to the left; i.e., Ca^{2+} potentiates low doses (1.0-1.7 g/kg) of ethanol. Finally, intracerebroventricularly-administered Ca^{2+} did not change blood ethanol levels compared with no cation treatment. This study expands the generality of Ca^{2+}-depressant drug enhancement reported earlier, and supports the evidence for involvement of a brain Ca^{2+} pool in behavioral intoxication in rodents. (Supported in part by USPHS, NIAAA Grant No. AA01417).

923 EFFECT OF MORPHINE ON SPONTANEOUSLY ACTIVE SINGLE UNITS OF CAT MYENTERIC PLEXUS. David N. Erwin, Toshiaki Ninchoji* and J. D. Wood. Depts. of Physiol. & Neurosurg., KUMC, Kansas City, Kansas 66103.

We recorded spontaneous action potentials extracellularly from neurons of cat Auerbach's plexus, using 20 micron teflon-coated platinum wires. Longitudinal muscle was removed from mounted sections of duodenum or jejunum to expose Auerbach's plexus, but the circular muscle, Meissner's plexus and submucous plexus were intact. A six minute control recording from each neuron was followed by six minutes of morphine sulfate at 1 to 5 x 10^{-5} g/ml final conc. in Tyrode's solution. In five experiments, Naloxone was added at 1 x 10^{-5} g/ml either before, during, or after the addition of Morphine. Types of cells recorded included mechano-sensitive neurons, bursting cells and single spike cells. Drug studies were only performed on bursters and single spike cells.

We measured the spike frequency of single spike cells in terms of the mean interspike interval. For burst cells, we measured the mean interspike interval (within bursts), the mean interburst interval, and the mean number of spikes per burst. The results are tabulated below:

29 Burst-Type Cells

Parameter	# Increase	# Decrease	# Unchanged
Mean Interspike Interval	8	19	2
Mean Spikes/Burst	6	16	2
Mean Interburst Interval	13	9	7

Columns indicating a change include only those experiments for which there was a significant difference according to Student's t-test between Control and Morphine. In most cases, the changes in interspike intervals were correlated with the appropriate change in spikes per burst. That is, the latter spikes in a burst tended to have the greatest interspike intervals; so that, if the number of spikes increased, the mean interspike interval also tended to increase.

We also recorded from five single spike cells. Three of these increased their spike frequency upon addition of morphine and two of the three were eventually blocked. Two single spike cells decreased their spike frequency upon addition of morphine. Naloxone had no detectable effect upon any of the cells at the dosage used.

In contrast to previously published work on the guinea-pig ileum, these results indicate that morphine has no consistent direct effect upon spontaneously active neurons of Auerbach's plexus in the cat. It is possible that the effects of morphine upon motility are mediated by other neurons not sampled by our recording methods.

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924 EFFECTS OF MORPHINE ON SELF-STIMULATION THRESHOLDS TO THE SUBSTANTIA NIGRA AND THE LOCUS COERULEUS IN THE RAT. R. Esposito* and C. Kornetsky*. (Spon: A. Peters). Boston University School of Medicine, Boston, MA 02118.

Acute administration of morphine in low to moderate doses (4mg/kg - 8mg/kg) has previously been found to reduce the threshold for intracranial self-stimulation (ICSS) to the medial forebrain bundle at the level of the lateral hypothalamus (MFB-LH) (Marcus and Kornetsky, Psychopharmacologia, 38: 1, 1974). Further, we have found no evidence that there is tolerance to this threshold lowering effect with chronic administration of morphine (Esposito and Kornetsky, Science, 195: 189, 1977).

Since the catecholamines have been implicated as critical substrates for both the phenomenon of ICSS (German and Bowden, Brain Research, 73: 381, 1974) and the self administration of rewarding drugs, particularly morphine (Davies and Smith, Life Sciences, 12: 185, 1973), it was decided to investigate whether morphine may lower or in other ways modulate ICSS thresholds to other "rewarding" brain sites, and to determine to what degree the morphine effect on MFB-LH stimulation may reflect differential catecholamine activity. Rats were implanted with bipolar stainless steel electrodes aimed at the substantia nigra (SN) or the locus coeruleus (LC) and trained on a modified method of limits to determine reinforcement thresholds from these brain sites.

At low doses (2mg/kg - 6mg/kg) morphine lowered the ICSS threshold to both the LC and the SN. Higher doses (8mg/kg - 12mg/kg) increased the threshold with the animals manifesting some degree of sedation. As with stimulation to the MFB-LH, there was no tolerance to the threshold lowering effect of the low dose in the SN animals. At the present time, the chronic administration of morphine to the LC animals has not been completed so that no statement regarding tolerance to these animals can be made at this time.

These data indicate that when rate-free measures of the reinforcing value of ICSS are employed, morphine will (within a narrow dose range) enhance the reinforcing value of stimulation to catecholamine rich areas of the brain. Further, and probably of most importance, to date we have failed to find tolerance to these threshold lowering effects.

These results suggest that the ICSS threshold lowering effects of morphine may represent a model for the study of the reinforcing properties of the narcotic analgesics.

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925 MORPHINE FACILITATION OF SHUTTLE-BOX SELF-STIMULATION IN THE RAT: FAILURE TO FIND TOLERANCE. Timothy M. Evers, Donald J. Stilwell*, and Robert A. Levitt. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901.

In a shuttle-box self-stimulation paradigm, a rat is able to control the onset and duration of rewarding electrical brain stimulation by crossing from one side of the cage to the other. Animals with lateral hypothalamic electrodes have been reported to leave the stimulation on about 10sec and off about 2sec per crossing. Systemic injections of an analgesic dose (10mg/kg) morphine were found to produce an immediate increase in average ON time per crossing (to about 20sec) without altering average OFF time. In this experiment we investigated the development of tolerance to this action of morphine.

One week after surgery, 16 adult rats, each implanted with one electrode in the lateral hypothalamic area, were tested in cages measuring 35x20x20cm, set on a fulcrum at the center, and with a microswitch at one end. Mean ON and OFF times per crossing during nine-ten minute periods per day were recorded on ten consecutive days. Days 1 and 2 were control days. On days 3-7, the 8 experimental animals were injected IP with 10mg/kg morphine sulfate immediately before placement in the shuttle-box. Days 8-10 were post drug control days. A second group of 8 animals received IP injections of isotonic sodium chloride solution on days 3-7.

ON times averaged about 8sec per crossing, while OFF times averaged about 2sec per crossing for both groups on training days 1 and 2. The shuttling behavior of the control animals did not change during the subsequent 8 days. On the first day of morphine injection, the experimental animals increased their mean ON times to about 25sec, but their mean OFF times remained near 2sec. This facilitative effect of morphine did not show tolerance over the subsequent 4 days of drug injections. ON and OFF times for the experimental group returned to baseline levels on day 8.

In the standard self-stimulation (SS) paradigm, morphine first depresses bar press rates for up to 3 hours and then facilitates SS for about 4 hours. With daily morphine injections tolerance develops to the initial suppression of SS but not to the later facilitation. The immediate facilitation of shuttle-box self-stimulation by morphine also does not show tolerance and seems analogous to the delayed facilitation found in bar-press SS. This action of morphine on shuttle-box self-stimulation may provide a useful model of the mood-enhancing effects of narcotic drugs in humans.

926 IONTOPHORESIS OF OPIATE ALKALOIDS AND ENDORPHINS ACCELERATES HIPPOCAMPAL UNIT FIRING BY A NON-CHOLINERGIC MECHANISM; CORRELATION WITH EEG SEIZURES. E. D. French*, G. R. Siggins, S. J. Henriksen and N. Ling*. The Salk Institute, La Jolla, CA 92037

In previous iontophoretic studies on anesthetized rats, normorphine and the opioid peptides Met^5-enkephalin (Met-enk) and β-endorphin generally inhibit neurons of most brain regions, while they markedly excite dorsal hippocampal (HPC) neurons (Nicoll et al., PNAS, 1977, in press). HPC excitations are also seen with iontophoresis of $(D\text{-}Ala^2)\text{-}Met^5$-enk amide, γ-endorphin and s.c. morphine (50 mg/kg). On rare occasions the excitations of HPC cells to iontophoretic β-endorphin are accompanied by large (2-5 mv) voltage deflections (interictal spikes) in d.c. microelectrode recordings. In agreement with iontophoretic studies, subcortical EEG recordings from HPC show dramatic non-convulsive seizures 1-2 min after intraventricular injection of 1-10 nM of morphine, Met-enk or β-endorphin; such seizures are blocked by naloxone (Henriksen, this vol.). These seizures are also associated with large (3-20 mv) interictal spikes in recordings from single-barrel micropipettes (1μ tip size), in correlation with the unit HPC excitations to iontophoresis of opiates.

The opiate excitations could result from presynaptic release of an excitatory neurotransmitter, such as acetylcholine (ACh) derived from cholinergic septal-HPC pathways. Although iontophoretic ACh excites HPC neurons, excitations to normorphine, Met-enk and β-endorphin are not diminished by iontophoretic currents of atropine or scopolamine sufficient for total blockade of ACh. Conversely, the opioid excitations, but not ACh responses, are blocked by iontophoretic or s.c. naloxone (2-8 mg/kg). Moreover, electrocoagulation lesions of medial septum, the origin of cholinergic fibers to the dorsal HPC, do not diminish excitations of dorsal HPC neurons to β-endorphin, morphine or Met-enk. This correlates with EEG recordings showing persistence of β-endorphin HPC seizures after i.p. scopolamine (4 mg/kg). These results support the hypothesis that opiate-induced excitations and seizures in HPC are independent of ACh release.

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927 ENKEPHALIN-INDUCED CORTICAL SEIZURES IN THE RAT. Hanan Frenk, Gideon Urca, and John C. Liebeskind. Dept. Psych., Los Angeles, CA 90024.

We have recently reported (Urca et al., Science, in press) that intracerebroventricular (ICV) injections of 200 μg met-enkephalin into the lateral ventricle induced powerful cortical epileptic activity in the EEG of rats. These injections also produced potent but short-lived tail-flick analgesia in 50% of the animals. In the present study we compared the effects on cortical EEG of ICV injections of met-enkephalin, leu-enkephalin, and morphine, all administered in a dose of 100 μg in 10 μl of Ringer's. In these quantities all substances elicited pronounced and morphologically similar seizure activity, which was blocked or greatly attenuated by the prior intraperitoneal injection of 10 mg/kg naloxone. It was noted that the duration of the seizures caused by leu-enkephalin (median = 13 min) was significantly longer than that caused by met-enkephalin (median = 6 min). The convulsant potency of enkephalin was underlined by the finding that ICV injections of doses as low as 25 μg leu-enkephalin still reliably caused seizures.

At doses of 100 μg, ICV injections of morphine elicited powerful and long-lasting tail-flick analgesia, but analgesia was only rarely observed after injections of the enkephalins at this same dose.

In a separate study, 120 μg of met-enkephalin in 1 μl of Ringer's was injected directly into, or in the immediate vicinity of the periaqueductal gray matter of rats. Seizures were not observed in any of these animals. Analgesia was found in those rats that were injected into sites ventral, but not dorsal to the aqueduct. On the basis of these experiments it was concluded that both the epileptic and analgesic effects of the enkephalins and morphine are mediated by opiate receptors. It seems likely, however, that the analgesic effect is mediated by receptors in the midbrain, whereas the EEG effect is mediated by receptors in other anatomical locations, most likely in the forebrain. A study aimed at locating these receptor sites is currently in progress.

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928 IN VITRO EFFECTS OF PHENCYCLIDINE ON RAT STRIATAL TYROSINE HYDROXYLASE AND ADENYLATE CYCLASE. Richard E. Garey, Carl W. Christensen,* Andrew D. Howard* and Morris A. Spirtes. Depts. of Psych. & Neurol., Physiology and Pharmacology, Tulane School of Medicine and Veterans Administration Hospital, New Orleans, La. 70112.

Utilizing whole brain homogenates, Domino and his colleagues (Arch. int. Pharmacodyn. 202: 252-258, 1973) concluded that phencyclidine (PCP) caused a relative lowering of catecholamine (CA) synthesis and an increase in CA metabolism. Further data by this group indicated that the biochemical steps from dihydroxyphenylalanine (DOPA) onward in the synthesis of CA were probably not involved. Thus tyrosine hydroxylase (T-OH) activity or substrate availability for this enzyme could have been altered by the drug. We now present evidence that PCP at 10^{-7} M stimulated T-OH activity in the striatum by at least 30% so that the statistically significant lowering of dopamine and norepinephrine found by the original group must have been due to an even greater increase in CA metabolism. This interpretation is supported by the finding of Garey and Heath (Life Sci. 18: 1105-1110, 1976) that PCP is a potent, competitive inhibitor of CA reuptake in the striatum. Such an inhibition could lead to the increase in O-methylated metabolites previously reported by the Domino group. In addition, we have found another effect of PCP, also in the striatum, namely, that it stimulates brain adenylate cyclase (AC) directly by at least 60% at a concentration of 10^{-8}M; i.e. a concentration 10x smaller than that required for T-OH stimulation. The question now is raised as to whether the AC effect is the primary action of PCP. This drug-induced increase in AC activity would also help in explaining the increased T-OH activity which we found. The latter could be due to activation of the T-OH enzyme via a phosphoprotein-kinase, a possibility suggested by Roth et al. in 1975 (In: Pre- and Post Synaptic Receptors ed. Usdin and Bunney, Marcel Dekker, Inc., New York. p. 18).

929 POTENTIATION OF MORPHINE BUT NOT MEPERIDINE ANALGESIA FOLLOWING SEROTONIN (5HT) REUPTAKE INHIBITION. G.F. Gebhart and S.A. Lorens. Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

The hot plate (55±0.5°C) and tail flick (60±0.5°C) methods were used to determine the effects of a new selective 5-HT uptake inhibitor, Zimelidine (Ross and Renyi, Neuropharmacol., 1977, 16: 57), on morphine and meperidine analgesia. Control paw lick (60 sec. maximum) and tail flick (15 sec. maximum) latencies were obtained. The rats (n=42; 258-345 g.) then received Zimelidine (Z; 10 mg/kg) or saline (S; 1 mg/kg) i.p., followed 1.5 hr. later by morphine sulfate (MOR; 3.0 mg/kg, as the base), meperidine HCl (MEP; 10.0 mg/kg, as the base) or saline (S; 1.0 ml/kg) s.c., and were retested 30 min. after the second injection.

No group differences in control response latencies were found. The post-injection latencies of all MEP and MOR groups were significantly (Wilcoxan test) longer than control. Z did not affect paw lick or tail flick latency, but potentiated the analgesic effect of MOR as measured by the hot plate but not the tail flick procedure. In contrast, Z pretreatment reduced (46%) the analgesic effect of MEP. The response latencies of the S-MEP and Z-MEP groups, however, did not differ significantly (see Table).

The effect of Z on meperidine HCl (MEP-24; 24 mg/kg, as the base) analgesia was re-examined in a new group. The schedule was as above, except that only the hot plate test (120 sec. cut off) and 2 groups were employed. The paw lick latencies (sec.) of the S+MEP-24 group (n=6) were significantly (T=0) longer post-injection (control, 7.4±0.9; post-drug, 60.3±20.1). In contrast, the pre- and post-injection response latencies (control, 9.1±2.0; post-drug, 30.5±11.8) of the Z+MEP-24 group (n=7) did not differ significantly ($T=2\frac{1}{2}$). Z thus produced a 49% reduction in paw-lick latency. The two groups, however, did not suffer significantly (U=11), primarily because of the variance observed.

5-HT uptake inhibition following Zimelidine injection can potentiate morphine analgesia but attenuate meperidine analgesia, suggesting that different 5-HT systems may be involved in mediating the analgesic effect of morphine and meperidine.

Mean (± S.E.M.) Response Latencies (Sec.)

	Paw Lick		Tail Flick	
Treatment	Saline	Zimelidine	Saline	Zimelidine
Saline	9.2±1.3	11.3±1.3	4.2±1.9	3.2±0.5
Morphine	14.3±2.1*	26.6±6.0**	10.7±2.1†	13.0±1.3†
Meperidine	26.6±8.3*	14.5±2.9	11.4±1.7†	9.1±1.6†

*Differs from S-S groups ($p \leq 0.05$, one-tail U test); **Differs from both S-S and other Z-treated groups ($p \leq 0.004$, two-tail) as well as S-MOR group ($p < 0.015$, two-tail); †Significant difference from S-S and S-Z groups ($p \leq 0.03$ one-tail). [NS 12114]

930 DIFFERENTIAL EFFECTS OF NARCOTICS AND RELATED COMPOUNDS ON THE FLUROTHYL SEIZURE THRESHOLD IN RATS. E.B. Geller*, A. Cowan*, B. Melamed* and M.W. Adler (SPON: R.C. Truex). Temple University School of Medicine, Philadelphia, Pa. 19140

Acute doses of morphine produce anticonvulsant effects in rats (Adler *et al.* JPET *198*:655,1976). To investigate the generality of this finding, we have compared the effects of several strong analgesics on the seizure threshold of rats challenged with flurothyl. Male, albino Sprague-Dawley rats (300-350 g; n=10-20) each received one of at least 3 doses of test compound s.c. 30 min prior to being exposed to flurothyl. The convulsant (a 10% solution in 95% ethanol) was infused onto a gauze pad fixed beneath the lid of a glass jar and the time to onset of a clonic convulsion with loss of posture was noted. Rats (n=50) pretreated with saline convulsed after 355 ± 5 (s.e.m.) sec. From the results, test compounds could be divided into 3 groups.

1. Compounds producing dose-related anticonvulsant effects: buprenorphine (0.0008-0.02 mg/kg; mean max. % increase in seizure threshold = 15%), levorphanol (2.5-20 mg/kg; 16%), morphine (12.5-50 mg/kg; 18%), phenazocine (0.50-5.0 mg/kg; 18%), N-allylnorphenazocine (SK&F 10,047) (10-40 mg/kg; 21%), and cyclazocine (1.0-5.0 mg/kg; 27%).

2. Compounds producing dose-related proconvulsant effects: meperidine (6.25-25 mg/kg; -12%) and pentazocine (12.5-50 mg/kg; -32%).

3. Compounds without dose-related effects: ketocyclazocine (0.50-5.0 mg/kg; 4%), ethylketocyclazocine (0.50-12.5 mg/kg; 5%), nalorphine (25-100 mg/kg; 10%), and ℓ-BL-4566 [(-)-3-cyclopropylmethyl-8-hydroxy-11-α-methoxy-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocine] (12.5-50 mg/kg; 7%).

It is of interest that doses of buprenorphine higher than 0.02 mg/kg (*i.e.* 0.10-12.5 mg/kg) had slightly less effect (range 7% - 10%) on seizure thresholds. Also, optimal anticonvulsant doses of cyclazocine (2.5 & 5.0 mg/kg) and SK&F 10,047 (20 & 40 mg/kg) caused head swaying and trembling of the forepaws. This behavioral syndrome was likewise observed after ℓ-BL-4566 (25 & 50 mg/kg) but was not associated with a marked increase in seizure threshold.

We conclude that a) the anticonvulsant action previously reported for morphine is shared by at least 3 other strong analgesics; certain psychotomimetic narcotic antagonists, at doses causing bizarre behavioral patterns in the rats, are also anticonvulsant, b) benzomorphan analgesics can have either pro- or anti- convulsant actions or no effect, c) three compounds, ethylketocyclazocine, ketocyclazocine, and nalorphine, presumed to have little or no intrinsic activity at so-called μ receptors (Martin *et al.* JPET *197*:517,1976) do not markedly influence seizure threshold. (Supported by grant DA 00376 from NIDA).

931 INTRAVENOUS SELF-ADMINISTRATION OF MORPHINE BY RATS: BEHAVIORAL FEATURES OF ADDICTION AND WITHDRAWAL. Philip L. Gildenberg, K.S. Krishna Murthy, Kevork G. Chatmajian* and Martin W. Adler. Div. Neurosurg., Univ. Tex. Med. Sch., Houston, TX 77030 and Dept. Pharm., Temple Univ. Sch. Med., Philadelphia, PA 19140.

Chronic intravenous self-administration of morphine by male Sprague Dawley rats (200-225 grams) was achieved after a 4-day schedule employing automatic hourly infusions of morphine sulfate (in a volume of 0.2 ml.) through a cannula inserted into the right external jugular vein. The cumulative daily dose of morphine during the 4-day schedule was 48, 72, 120 and 192 mg./kg. respectively. On day 5 the infusions were programmed for self-administration (activated by pressing a lever by the animal) on an FR 1 schedule for 5 days and then an FR 3 schedule. After 5 days on the FR 3 schedule the rats were subjected to abrupt withdrawal by substituting Ringer's solution for morphine. Behavioral features exhibited during the period of withdrawal were measured 24 and 48 hours after abrupt withdrawal.

The lever presses and infusions were tabulated. The body weight of each animal was monitored daily. Rats which self-administered morphine tended to press the lever considerably more frequently than necessary for the number of infusions recorded. A difference in the means of the daily lever presses of the morphine group compared to the control group became significant at the 0.05 level on the third day of self-administration and at the 0.01 level the fourth day of the FR 1 schedule. The significance increased to the 0.005 level after the start of the FR 3 schedule. Withdrawal symptoms were more pronounced at 24 hours when compared to 48 hours after abrupt withdrawal of morphine. The most prominent symptoms of abrupt morphine withdrawal were teeth chatter, diarrhea, rapid breathing, ptosis and loss of body weight (mean 14.9% ± 3.4 S.D.).

932 CARDIOVASCULAR AND VENTILATORY EFFECTS OF METHIONINE AND LEUCINE ENKEPHALINS ADMINISTERED BY INTRAVENOUS OR CLOSE-ARTERIAL INJECTION IN THE CAT. A.H.Hassen and M.K.Kindred*. Div. Allied Health & Life Sciences, Univ. of Texas, San Antonio, TX 78285.

Enkephalins are endogenous ligands for the morphine receptor. The effects of systemically or locally administered enkephalins upon various physiologic parameters have been examined in a variety of animal species. These studies have demonstrated similarities and differences in the responses to enkephalins as compared with the responses to morphine. As part of an on-going study of the actions of narcotics on brainstem regulation of autonomic activity, we have examined the cardiovascular (CV) and ventilatory (VENT) responses produced by MET- and LEU-enkephalin. In order to describe in a more precise manner the nature of enkephalin activity, these compounds were administered both systemically, and locally to the brainstem. All experiments were performed on decerebrate cats. Ventilation was monitored by means of a pneumotachograph connected to a tracheal cannula, providing direct measurements of ventilatory flow and rate. Tidal volume was derived from the integrated flow using a Narco GPA-10 Integrator. Blood pressure was monitored from the RT femoral artery. Systemic injections were made via an indwelling cannula, into the RT femoral vein. Close-arterial injections were made via an indwelling cannula into the subclavian artery, with all branches except the vertebral ligated. Systemic administration of enkephalins (2.0mg/kg) produced a drop in both diastolic and systolic blood pressures. These changes frequently were associated with bradycardia. VENT responses were variable among the different animals. In general, CV responses were much more consistant than VENT responses. All responses were of short duration (less than 5 minutes) and could be blocked by prior administration of Naloxone (0.2mg/kg). Close-arterial injection of enkephalins (0.25-1.0mg/kg) produced responses comparable to those observed following systemic injection, but at 12% to 50% of the systemic dose. CV responses were again more consistant and of longer duration than VENT responses. While the injection of enkephalins consistantly produced a predictable CV response, with morphine the predictable response was ventilatory.

In conclusion, it is noted that enkephalins alter CV and VENT activity in the cat, in a manner different from morphine, and appear to do so primarily as a result of an effect on central brainstem mechanisms.

933 INDUCTION OF LIMBIC SEIZURES BY ENDORPHINS AND OPIATE ALKALOIDS: ELECTROPHYSIOLOGICAL AND BEHAVIORAL CORRELATES. S. J. Henriksen, F. E. Bloom, N. Ling* and R. Guillemin. The Salk Inst., La Jolla, CA 92037.

Rats were prepared with cortical and subcortical electrode arrays and chronic indwelling ventricular cannulae. Following at least 3 days recovery from surgery, beta-endorphin (BE), ($D\text{-}Ala^2$)-Met^5-enk amide (DAME), a potent enkephalin derivative, and opiate alkaloids all precipitated complex limbic seizures in unrestrained rats when injected in nanomolar (nM) doses. Beta-endorphin (5-10 nM) precipitates multiple ictal spiking episodes (duration 30-90 sec.), starting 90 sec. to 2 min. following the injection. During these ictal episodes high frequency spike discharges are observed simultaneously in the dorsal hippocampus, amygdala, and other limbic loci. Behaviorally, the animals maintain a frozen posture, stare, chew, and occasionally orient rapidly, but no frank convulsions are observed. The ictal episodes are also punctuated by violent shaking of the torso ("wet-dog-shakes"). Following the initial ictal episodes, animals show increased exploration with constant sniffing. Multiple ictal episodes are followed by tonic inter-ictal spike activity in the amygdala and hippocampus at a spike frequency of 10-20/min. Fifteen to thirty min. post injection high amplitude cortical slow-waves dominate the EEG, and analgesia (corneal reflex and tail pinch) develops with catatonia soon ensuing. Inter-ictal spiking continues during the entire catatonic episode lasting for up to 6 hrs. Lower doses of BE (< 3 nM) produce little or no analgesia nor catatonia, but ictal and inter-ictal spiking episodes persist. Using equimolar (3×10^{-9}M) doses, BE produces the most prolonged seizure episodes with DAME-amide and morphine sulfate producing correspondingly less potent effects. Naloxone (1-2 mg/kg, I.P.) results in a rapid but temporary reversal of the behavioral and electrographic signs. These results suggest that opiates and endorphin peptides produce profound and lasting electrophysiologic disturbances in limbic structures at doses below the threshold for their anti-nociceptive actions.

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934 NALOXONE EXCITATION OF DORSAL HORN UNITS IN THE SPINAL CAT. James L. Henry. Dept. of Research in Anaesthesia, McGill University, Montreal, P.Q. Canada. H3G 1Y6

Naloxone has been shown to have actions in the central nervous system independent of those as an antagonist of centrally or systemically administered opiates. This has led to the suggestion that these actions are due to an antagonism of endogenous opioid substances, likely peptides, which are being released continuously. Microiontophoretic studies have demonstrated an action of opiates and of endogenous opioid peptides in the spinal cord, and other studies have revealed the abundance in the spinal cord of stereospecific "opiate receptors". Thus the endogenous opioids may be released in the spinal cord and depress activity in nociceptive pathways. The following investigation was done therefore to see whether naloxone modifies the activity of spinal nociceptive neurones. Cats were anaesthetised with alpha-chloralose (60 mg/kg). The spinal cords were transected at the level of the first lumbar vertebra and segments L5-L7 were exposed for recording. Extracellular unit spikes were recorded using glass micropipettes filled with 2.7 M NaCl. Single units responding in a reproducible way to regular periodic applications of noxious radiant heat were selected for study. The intravenous administration of naloxone (0.05-0.1 mg/kg) caused an increase in the on-going discharge rate of all units tested. This effect began within one minute, reached its maximum at three to five minutes and persisted for more than one hour. In addition, naloxone facilitated the response to noxious heat. This facilitation followed a similar time course to the increase in ongoing activity. These observations support the suggestion that an endogenous opioid is being continuously released in the spinal cord which reduces transmission of nociceptive information at this level. However, these experiments do not exclude the possibility that naloxone itself may be causing excitation in spinal nociceptive pathways, perhaps as an opiate agonist.

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935 AMPHETAMINE-ETHANOL INTERACTION ON ACTIVITY AND MAZE PERFORMANCE IN RATS. Joan A. Holloway Dept. of Psychiatry and Behavioral Sciences, Univ. of Okla. Health Sciences Center, Oklahoma City, Oklahoma 73190.

The effects of single or combined doses of d-amphetamine sulfate and ethanol were studied in rats. Jiggle platform activity as well as photocell activity counts were measured simultaneously. Additionally, performance on a previously learned Y-maze-discrimination-avoidance-task was measured. Measurements were taken after drug administration at two times in the 24 hour light-dark cycle, at the peak circadian susceptibility to amphetamine and when susceptibility had fallen off sharply.

Results indicated a significant drug interaction as well as a significant drug by time-of-administration effect for activity measures. The results were different for the two activity measures. Photocell measurements were best at differentiating between ethanol and ethanol-amphetamine combinations and jiggle platform measurements were best at differentiating between amphetamine and ethanol-amphetamine combinations.

Performance measures further indicated a drug interaction. Performance was best with low-dose combinations of the drugs compared to single administration of the same drugs as reflected by number of correct avoidance responses and start latencies.

These data indicated that the drug interaction between ethanol and amphetamine was not antagonistic but synergistic at low doses and the extent of the interaction depended on the time in the light-dark cycle the drugs were administered.

936 INCREASE IN STRIATAL METHIONINE ENKEPHALIN CONTENT CAUSED BY CHRONIC ADMINISTRATION OF CATALEPTOGENIC ANTIPSYCHOTICS. J. S. Hong*, H.-Y. T. Yang*, W. Fratta* and E. Costa. Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hosp., Washington, D.C. 20032.

In an attempt to obtain some indication on the regulation of met-enkephalin containing neurons by other neuronal systems, antipsychotic drugs were chronically administered to rats for 7 to 21 days and met-enkephalin content of striatum and hypothalamus was determined by radioimmunoassay. Acute administrations of haloperidol (1 mg/kg) failed to alter met-enkephalin levels. Following chronic treatment with haloperidol (1 mg/kg, twice daily), the met-enkephalin concentration in striatum gradually increased from 10 ng/mg protein (control) to 14 ng/mg (one week), 17 ng/mg (two weeks) and 20 ng/mg (3 weeks). In the same rats, neither hypothalamic met-enkephalin nor striatal substance P content was changed. This supports the possibility that the increase in striatal met-enkephalin content elicited by haloperidol is specific. An increase of met-enkephalin concentration in striatum was also observed after two weeks chronic administration of pimozide (0.75 mg/kg) or chlorpromazine (3.0 mg/kg). On the other hand, chronic treatment with a non-cataleptogenic antipsychotic, clozapine (5.0 mg/kg), failed to change striatal met-enkephalin content. The selective increase of striatal met-enkephalin content which appears after chronic, but not acute, haloperidol treatment and fails to appear after chronic treatment with a non-cataleptogenic antipsychotic suggests that a relationship may exist between tolerance to extrapyramidal side effects, supersensitivity of DA receptors to the agonist and striatal met-enkephalin content.

937 β-ENDORPHIN: DEVELOPMENT OF TOLERANCE AND ITS REVERSAL BY 5-HYDROXYTRYPTOPHAN IN CAT. Y. Hosobuchi*, J. Meglio*, and C.H. Li*. Dept. of Neurosurg. and Hormone Research Lab., Univ. of Calif., San Francisco, CA 94143.

β-endorphin is a potent analgesia when administered intraventricularly (icv) in cat. Repeated injections of this peptide produce acute tolerance, as well as cross-tolerance to morphine. This study was undertaken to analyze the development of tolerance to β-endorphin in cat, and the reversal of tolerance by systemic administration of the serantonin precursor, 5-hydroxytryptophan (5-HTP). Synthetic β-endorphin was administered icv through stereotactically placed third-ventricle cannulas. The extent or absence of analgesia was determined by stimulating tooth pulp and measuring the jaw opening reflex (JOR), and by observing the response to pinches applied with a tooth forceps to the cat's tail, limbs, and ears. In all cats, 25 μg β-endorphin icv was the minimum effective dose (MED) required for analgesia. Acute tolerance was observed when administration of β-endorphin was repeated within the first 24 hr, even if the initial dose administered was below MED (12.5 μg) or if the second was above MED (50 μg). The analgesic effect returned to its original potency if the second dose was administered after 48 hr.

In four cats, 50 μg β-endorphin was injected icv within 24 hr after an initial injection of 25 μg (MED); no analgesic effect was observed, although behavioral alterations occurred. Tests made for 1 hr after the second injection confirmed that there was no delayed analgesic response. 5-HTP (10 mg/kg) then was administered intraperitoneally (ip) to these cats. Within 10 min, a marked analgesic effect was noted in the response of all four cats to JOR and peripheral pinch tests; the effect generally lasted 3-6 hr. When 5-HTP was injected 40-60 min after icv administration of 12.5 μg (below MED) β-endorphin, a 3- to 4-fold increase in JOR and decreased or absent responses to pinch were observed. The analgesic effect was totally reversed by naloxone.

These results confirm our previous observations that a single icv administration of β-endorphin produces acute tolerance to its analgesic effect in cat. They show that tolerance is reversed by systemic administration of 5-HTP, which also potentiates the analgesic effect of β-endorphin. Several investigators report that 5-HTP has similar effects in reducing development of tolerance to, and potentiating the analgesic action of, morphine in mice.

Since 5-HTP is known to elevate intracerebral seratonin content, our results suggest that analgesia produced by β-endorphin may be related to activation of a seratonergic pathway. If the first dose of β-endorphin produces reduction of seratonin in a given brain area, a second dose might be expected to be ineffective; its effect could be restored by administration of the seratonin precursor, 5-HTP.

938 STEREOSPECIFIC AND NON-STEREOSPECIFIC EFFECTS OF (+) AND (-) MORPHINE. Yasuko F. Jacquet, Werner A. Klee*, Kenner C. Rice* and Ikuo Iijima*. NY State Res. Institute for Neurochem., Ward's Isl., NY 10035, and NIMH and NIAMDD, Bethesda, Md 20014.

Unnatural (+)-morphine synthesized from natural (-)-sinomenine was evaluated in several standard opiate assay systems to ascertain whether all opiate actions are mediated by stereospecific receptors. In 3 in vitro assays, i.e., binding to rat brain homogenates, inhibition of adenylate cyclase activity in neuroblastoma x glioma hybrid cell homogenates, and inhibition of electrically-induced contraction of the guinea pig ileum, the unnatural (+)-morphine had no activity either as agonist or antagonist. Microinjection of 80 ug of (+)-morphine into the periaqueductal gray (PAG) of unanesthetized rats resulted in at most weak analgesia, and did not abolish the corneal reflex. However, the explosive motor behavior usually seen following microinjection of natural morphine was observed at this dose of unnatural morphine. This high dose of (+)-morphine added to a standard dose (10 ug) of (-)-morphine injected into the PAG, or naloxone given intraperitoneally (10-mg/kg) resulted in some deaths. Thus, the toxic effects of morphine in the PAG appear to be non-stereospecific. Microinjection of (+)-morphine into the midbrain reticular formation (a site previously shown to mediate potent and morphine-specific rotation of up to 2-3/sec (Jacquet, Carol and Russell, Science 192:261, 1976) which was not blocked, reversed or mimicked by naloxone injected locally or intraperitoneally) also resulted in a dose-dependent, non-naloxone reversible rotation behavior. These results indicate the existence in the CNS of at least 2 classes of opioid receptors, one which stereospecifically mediates analgesia and is reversible by naloxone, and another which is not stereospecific and not blocked by naloxone. Furthermore, the receptors assayed in the in vitro assay systems, i.e., brain homogenate binding assay, the cyclase inhibition assay and the guinea pig ileum assay, appear to be of the former class.

939 COCAINE LEVELS IN PLASMA AND RED BLOOD CELLS AFTER INTRAVENOUS AND INTRANASAL ADMINISTRATION IN MAN. J. I. Javaid, M.W. Fischman*, J. M. Davis, H. Dekirmenjian, and C. R. Schuster*. Ill. State Psychiatric Inst., Chicago, Ill. 60612 and University of Chicago, Ill. 60637

Cocaine is a short acting central stimulant, the pharmacology of which has not been studied in man. In the present studies cocaine Hcl was inhaled intranasaly (i.n.) or dissolved in physiological saline and injected intravenously (i.v.) over a period of one minute to adults with a long history of illicit intravenous cocaine use. All subjects signed a consent form which indicated that psychomotor stimulant drugs would be administered. Blood samples were withdrawn at different time intervals following different doses of drug administration and processed immediately for cocaine levels. Cocaine was determined by gas chromatography using an electron capture detector. Systolic and diastolic blood pressure were monitored on an Automatic Blood Pressure Monitor. Subjective effects of the drug were measured by the stimulant sections of the Addiction Research Center Inventory (ARCI) questionnaire and the Profile of Mood Scales (POMS). Cocaine levels were correlated to subjective and physiological responses.

Peak plasma and RBC levels were reached in five minutes after i.v. administration with RBC to plasma ratio of greater than one. Peak plasma levels after 16 mg i.v. administration were 221±41 ng/ml (n = 12) with a range of 120-586 ng/ml. After 32 mg i.v. administration the peak plasma levels were 283±23 ng/ml (n = 8) and ranged between 207-409 ng/ml. Increase in heart rate, blood pressure and subjective ratings was dose related although the onset of action did not have any relation to dose. There was a positive relationship between plasma levels and subjective ratings at both doses.

The individuals with higher peak levels at a given dose generally gave higher subjective response compared to the subjects who had lower peak levels at the same dose.

Peak plasma levels after i.n. administration of cocaine were reached in 10 to 60 minutes and ranged between 21-75 ng/ml at 16 mg dose; 106-276 ng/ml at 32 mg dose; 181-195 ng/ml at 64 mg dose and 129-296 ng/ml at 96 mg dose. The time of peak plasma level after i.n. administration correlated with the peak level of subjective response. Significant amounts of cocaine were present at higher doses even 2 hours after i.n. administration (156±64, n=2, at 96 mg dose).

940 LONG TERM SUPPRESSION OF NARCOTIC DEPENDENCY IN THE PRIMATE. Frederick W. L. Kerr. Dept. Neuro. Surg., Mayo Foundation, Rochester, MN 55901.

Macaque monkeys maintained in restraint chairs were trained to self-administer morphine (M.S.) by bar pressing on a fixed ratio of 10:1; each cycle provided 10 mg M.S. via a spring loaded self-refilling syringe connected to a right atrial catheter. They also obtained 1 gm food pellets on a 10:1 ratio. Dependence on morphine reached levels which were relatively stable for individual animals of 250 to 500 mg/day or approximately 60 to 125 mg/Kg B.W./day.

Intense withdrawal signs, graded on a 0 to 4+ scale, were precipitated by administration of naloxone, or by withholding morphine.

Fine cannulae were then implanted stereotaxically and under X-ray control in the lateral hypothalamus bilaterally and 7 to 10 days later 6-OHDA administered in doses of 50 to 125 μg on each side.

In all instances in which the cannulae were correctly positioned a pronounced fall in morphine demand occurred. However, to obtain complete suppression it was necessary to vary the position of the cannulae over a vertical distance of 2 to 2.5 mm and administer 6-OHDA on repeated occasions, demand for morphine decreasing in a stepwise manner with each successful injection.

Demand for food pellets dropped in parallel with that for narcotics, but after 2 to 3 days of anorexia, recovered progressively and returned to normal levels.

Complete suppression of narcotic dependence has been obtained in two monkeys, the effect persisting at 4 and 9 months after the lesions were completed. Both animals appeared to be neurologically intact and showed normal behavioral patterns when placed in a group situation. Growth hormone and prolactin levels in blood were within the normal range. Reduction of dependence to less than 10% of prelesional levels was obtained in other monkeys. Whether the 6-OHDA effect is specific or non-specific is uncertain in view of the doses employed.
(Supported by Grant DA 00110.)

941 MORPHINE EFFECTS ON THREE TYPES OF AVERSIVE MIDBRAIN STIMULATION IN RATS. R.S. Kiser, D.C. German, and R.M. Lebovitz. Depts. of Psychiat. & Phys., Univ. of Texas Health Sci. Cntr., Dallas, TX 75235.

The present study examined the effects of morphine and its antagonist, naloxone, on decremental bar-pressing performance in rats stimulated at three aversive midbrain sites. The three types of aversion can be differentiated on the basis of stimulation site and the elicited gross behavior. One type of aversion is produced by stimulation in the dorsal central gray (DCG) area. Stimulation here produces frantic rearing, jumping, and running, along with attempts to escape the behavioral chamber. Another aversive site is located in the ventral reticular formation (VRF), ventrolateral to the central gray area. VRF stimulation induces a stereotyped circling behavior, usually ipsiversive. A third type of aversive behavior is elicited from the dorsolateral tegmentum (DLT). DLT stimulation produces wincing, shaking, and shuddering behavior often accompanied by whimpering and crying. Rats stimulated at these sites can be trained to bar press for escape in a decremental bar-pressing paradigm. In this paradigm, each bar press decrements the stimulation current by 5% of its initial level. Rats were implanted with a bipolar stimulating electrode in one of the aversive midbrain sites described above and trained in decremental bar pressing. The brain stimulation consisted of 100 msec. duration trains of negative square wave pulses (0.5 msec. pulse duration, 60 Hz, capacity coupled) at the rate of 5 trains per second. Mean current intensities at all 3 sites was approximately 10-15 μa, RMS. Immediately after acquisition of baseline decremental bar-pressing performance, DCG, VRF, and DLT rats were injected intraperitoneally with either morphine (15 mg/kg), naloxone (2 mg/kg), morphine (15 mg/kg) and naloxone (2 mg/kg), or normal saline. The effects of the injections on decremental bar-pressing performance were assessed in data runs one hour later. In subsequent experiments separated by at least one week, all animals eventually received all of these injections in random order. No change in bar-pressing performance was seen in the saline-injected control animals. Morphine given alone inhibited decremental bar pressing in all three classes of animals, and naloxone alone had no effect. Naloxone given with morphine, however, blocked the inhibitory effect of morphine in all three groups. At no time were signs of impairment of motor function or level of consciousness observed. These results suggest that the antinociceptive effects of opiates are complex and involve multiple aversive neural systems.

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942 OPIATE-LIKE, NALOXONE-REVERSIBLE EFFECTS OF ANDROSTERONE SULFATE IN RATS. F. S. LaBella, V. Havlicek, C. Pinsky* and L. Leybin* Dept. of Pharmacol. and Ther. and Dept. of Physiol., Univ. of Man. Fac. of Med., Winnipeg, Canada, R3E 0W3.

Androsterone, androstan-5a-3a-ol-17-one (A), androsterone-3-SO_4 (AS), and some related compounds show weak binding (IC_{50} = 10^{-5}M) to the opiate receptor, in contrast to 150 steroids which are completely inactive. The in vitro Na effect indicates A to be an antagonist and AS an agonist. AS was infused over 5 min in 10 ul of 10% ethanol in saline to male rats via intracerebroventricular (ICV) cannulae with simultaneous EEG recording. The vehicle was inactive even at 25 ul. Less than 1 ug AS (3 nmoles) was threshold for EEG changes and slight behavioral effects. 3 ug caused EEG seizure activity in all animals and motor seizures in some; this dose also caused Straub tail, wet dog shakes, teeth chattering, circling, hypermotility, and hyperreactivity to auditory and tactile stimuli. 10 and 25 ug AS in most animals caused violent tonic seizures, and escape behavior and extreme aggression persisting for several hours. 5 and 10 ug promoted analgesia in the hot plate test. These effects of AS were reversed or blocked by naloxone (NAL). NAL given ICV inconsistently diminished drug-induced EEG or behavioral responses, and large doses were required to antagonize analgesia. However, pretreatment with NAL (5 mg/kg) given i.p. 15 min prior to AS consistently antagonized the steroid. Within 30 min after AS the NAL pretreated rats were asleep, with corresponding slow wave sleep or REM pattern of the EEG and having demonstrated little or no EEG or behavior changes. In contrast, saline pretreated animals showed the full blown opiate-like syndrome over the entire period of observation. This study supports our hypothesis that opiates and steroids share a common mechanism of action in the CNS (Life Sci. 16: 1783; 16: 1785, 1975). We find that 2 ug beta-endorphin given ICV produces similar behavioral and EEG effects but no motor seizures. Higher doses of the peptide, 10 and 50 ug, yield EEG hypersynchrony and catatonia rather than electrical and behavioral seizures seen with AS. Epiandrosterone, androstan-5a-3b-ol-17-one-3-SO_4 (ES), is considerably more potent than AS and promotes more violent seizures and aggression. NAL reversal of ES has not been tested. 10 ug of 17b-estradiol-3-SO_4 or androst-4-ene-3-one-17b-SO_4 were inactive. Results with AS resemble findings on somatostatin which shows weak in vitro but potent in vivo opiate activity and is blocked by NAL. AS may act on a family of receptors distinct from those binding recognized morphine-like compounds and/or it may mobilize endogenous opiates. (Supported by MRC, Non-Medical Use of Drugs Directorate, and the Sellers Foundation).

943 MORPHINE AND SHUTTLE-BOX SELF-STIMULATION IN THE RAT. Robert A. Levitt, John H. Baltzer, Timothy M. Evers, Donald J. Stilwell*, and John E. Furby*. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901

One interesting apparatus for the study of narcotic drug effects on SS is the shuttle-box. In this apparatus the animal moves back and forth across a cage to turn brain stimulation on and off. The animals not only self-regulate the time of onset of ON and OFF periods, but also the duration of such periods. This type of SS may be particularly sensitive to the interaction of the rewarding and aversive components of brain stimulation.

Adult rats served as subjects. Each animal was implanted with one electrode in the lateral hypothalamic area. Testing occurred in cages measuring 35x20x20cm, set on a fulcrum at the center, and with a microswitch under one end. Electrical stimulation was provided by a square wave stimulator. Beginning one week after surgery, animals were tested in the shuttle-box for 90 minutes a day on six consecutive days. Mean ON and OFF times during each of the nine-ten minute periods per day were recorded. On days 2, 4, and 6 additional control 90-minute self-stimulation tests were given in the shuttle-box. On days 3 and 5 animals received drug injections immediately before being placed in the shuttle-box. Half of the animals received a dose of morphine on day 3 and isotonic sodium chloride solution on day 5; for the other half of the animals this order of treatments was reversed. Eight animals each were run at the 1.0, 2.5, 5.0, 10.0, and 20.0 mg/kg doses.

Control ON times averaged about ten seconds, while OFF times averaged about two seconds. Sodium chloride injections, and the injections of 1.0 or 2.5 mg/kg morphine did not significantly affect the animal's shuttling behavior. Injections of 5.0 or 10.0 mg/kg morphine, however, increased average ON time, while having little effect on OFF time (90 minute average; 5 mg/kg: ON time = 21 seconds, OFF time = 2 seconds; 10 mg/kg: ON time = 21 seconds, OFF time = 2 seconds). Injections of 20 mg/kg produced an even further increase in average ON time (64 seconds), together with a similar large increase in average OFF time (70 seconds). These findings were confirmed statistically.

In this shuttle-box SS paradigm analgesic doses of morphine (5 or 10 mg/kg) produced a large increase in the amount of time the animals left the stimulation on, without affecting OFF times. When coupled with the evidence that animals turn off initially-rewarding brain stimulation because it becomes aversive, we wish to suggest the notion that morphine enhances brain stimulation reward by inhibiting its aversive component. This action of morphine on shuttle-box self-stimulation in the rat may also prove to be a useful model for the mood-enhancing action of narcotic drugs in the human.

944 SIGNAL DETECTION THEORY ANALYSIS OF NOCICEPTION IN RHESUS MONKEYS. Charles G. Lineberry and Albert T. Kulics. Dept. Pharmacol., Sch. Med., Univ. of Pittsburgh, Pgh. PA 15261

In previous work, we have demonstrated a signal detection theory (SDT) model for assessing sensory functions in rhesus monkeys, and evaluated this model in direct comparisons between human and monkey subjects engaged in the same experimental task. SDT analysis of discrimination performance provided relative operating characteristic (ROC) functions and sensory sensitivity estimates (d' and d'_e) which were virtually identical for both species. This approach was used to determine the effects of morphine, diazepam, local injections of lidocaine and the presentation of conditioned stimuli on responses to noxious electrical cutaneous stimulation in rhesus monkeys. The conditioned stimuli were designed to be analogous to the implicit instructions that accompany placebo administration in humans. Separate groups of subjects were trained to either discriminate between noxious stimulus intensities in a go, no-go discrimination task, or to rate stimuli according to noxiousness by responding to terminate stimulation in an intensity rating task. Binary decision probabilities and response latencies (used as certainty ratings) were used in the discrimination task, and escape probabilities and response latencies (used as intensity ratings) were used in the intensity rating task to construct ROC functions. These ROC functions were used to obtain estimates of pain sensitivity (d') and response bias (β).

Estimates of d' were obtained for both tasks which: a) reliably reflected stimulus intensity differences, b) varied little within subjects upon replications in control sessions, and c) were insensitive to large differences in response bias (overall response tendency) that were observed within and between subjects. Diazepam (.125 and .250 mg/kg), lidocaine (1 and 2%) and the conditioned placebo stimuli reduced the tendency for subjects to respond to noxious stimuli (i.e., produced a change in response bias). Morphine (.125 to .500 mg/kg) had minimal effects on response bias (in general slightly increased responding) and none on pain sensitivity. Only lidocaine was effective in reducing pain sensitivity.

These studies demonstrate that psychophysical measures of pain perception can be obtained in monkeys using SDT techniques that: a) are directly analogous to those used in human subjects, and b) are capable of distinguishing manipulations which affect pain perception from those that affect only the response to pain.

945 UNIT RESPONSES OF MEDIAL THALAMUS TO LOW DOSES OF MORPHINE AND NALOXONE IN DRUG-NAIVE AND MORPHINE-DEPENDENT RATS.
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Previous lesion (Teitelbaum et al., Science 185, 1974) and chemical stimulation studies (Wei et al., Science 177, 1972) have pointed to the possible importance of the medial thalamic area in morphine tolerance and physical dependence. In the present study, the spontaneous activity of medial thalamic units was recorded prior to and following an i.v. injection of 0.625 mg/kg morphine (M) in drug-naive, chronically-prepared, paralyzed rats. Previous experiments established this dose to be near threshold for producing a change in spontaneous firing rate. Of the 15 units studied successfully, 14 showed a decrease in rate in response to morphine with one showing no change. This decrease in firing rate generally began to occur about one minute following the beginning of the drug injection (duration=30 sec.), with recovery beginning between 10 and 30 min. post-injection. In 13 of the 15 units, a decrease was evident prior to appearance of spindle-like activity in the simultaneously recorded fronto-cortical EEG. A dose of 0.1 mg/kg naloxone, which was sufficient to reverse morphine-induced depression, by itself had no effect on unit rate. In the case of 3 units, a second injection of the same dose was given following recovery from the first. A smaller decrease in firing rate and a shorter duration of response indicated that some tolerance had developed to the drug.

When the unit responses to 0.625 mg/kg M were compared to those of 8 previously recorded responses to 5 mg/kg M, it was found that while the maximum decrease in unit rate was not different, the degree to which the EEG changed was greater at the higher dose. This suggests that the medial thalamic units were near maximally affected at 0.625 mg/kg M, and that additional elements were affected at the higher dose to contribute to the increased EEG response.

Preliminary data from morphine-dependent animals (1-75 mg pellet implanted s.c. for 3 days), indicates that the threshold dose of naloxone required to produce an increase in medial thalamic unit activity is near 0.025 mg/kg. However, doses below this have nevertheless been effective in reversing the high amplitude fronto-cortical EEG to continuous fast activity, suggesting that at least this effect might be mediated by factors other than a change in frequency of medial thalamic units.

946 EFFECTS OF PAIN, AND THE IONTOPHORESIS OF MORPHINE AND ACETYLCHOLINE ON NEURONS IN RAT NUCLEUS RAPHE MAGNUS.
Michael Lobatz*, Herbert K. Proudfit and Edmund G. Anderson. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

The involvement of the nucleus raphe magnus (NRM) in pain and opiate analgesia is indicated by the blockade of morphine (M) analgesia following destruction of the NRM in the rat (Proudfit and Anderson, Brain Res. 98, 612, 1975). In examining nocisponsive neurons in the cat NRM we observed that iontophoretically applied M did not alter unit nocisponsiveness or baseline spontaneous activity, but systemic opiates did affect activity (Lobatz et al., Pharmacologist 18, 213, 1976). These findings suggest that, in the cat, M does not act directly on the NRM. To determine if NRM neurons in the rat react to pain and opiate analgesics in a similar manner, spontaneously active NRM units of intact, urethane-anesthetized rats were recorded, and frequency changes were measured following a painful pinch and iontophoretically applied M. Seventy-four % of the tested units responded to pinch. Of those responding, 87% were facilitated and 13% were inhibited. Morphine (25-100 nA) altered baseline activity in 61% (of those altered, 57% were facilitated and 43% were inhibited) and blocked pinch in 47% of the nocisponsive NRM units. These data show that rat NRM neurons are affected by noxious stimulation and the direct application of M. The effect of M is in contrast to data previously obtained in the cat and may reflect a species difference. Since acetylcholine (ACh) may play a role in M analgesia (see review by Mehta, Neuropharmacology 14, 893, 1975), we examined the effects of iontophoretic ACh on rat NRM neurons and its interaction with M. It was observed that iontophoretically applied ACh excited 85% of the nocisponsive NRM neurons, but the application of M did not block the ACh response. These data indicate that the NRM cells are highly responsive to ACh, and that M does not block this action. (Supported by USPHS Grant NS 12649.)

947 ROLE OF THE BLOOD-CEREBROSPINAL FLUID BARRIER IN THE DEVELOPMENT OF TOLERANCE TO THE RESPIRATORY DEPRESSANT EFFECTS OF MORPHINE.
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Tidal volume, respiratory rate, and minute volume were measured during continuous intravenous infusion of morphine sulfate (0.4 mg/kg/min) in morphine tolerant (10 day incrementing dose schedule reaching 400 mg/kg/day), naloxone pretreated (0.5 mg/kg, IV), and naive adult albino rats. After ten minutes of intravenous infusion, naive controls showed a depression in respiratory function while tolerant and naloxone pretreated animals were resistant to this morphine-induced effect. Respiratory depression in naive animals appeared to be regulated via a transport site in the choroid plexus which allowed nanomole amounts of morphine to penetrate into CSF and then by diffusion, into periventricular opiate receptors in brain stem. Tolerant rats showed a blockade of morphine-induced respiratory depression associated with a 48% decrease in the rate of penetration of the opiate into both CSF and brain stem. A similar pharmacologic change was not found in naloxone-pretreated animals, although complete blockade of morphine-induced respiratory depression was observed. Although tolerance to the respiratory depressant effects of morphine was completely abolished by intraventricular infusion, showing a profile similar to that seen in naive controls, this direct route of opiate administration failed to depress respiratory function in animals pretreated with naloxone.

In conclusion, evidence has been obtained suggesting that tolerance to the CNS respiratory depressant effects of morphine may be mediated, at least in part, by active transport sites regulating penetration of the drug from plasma into the CSF system, thereby decreasing access of nanomole amounts of the opiate to receptor sites of respiratory neurons located near the subependymal zone at the floor of the IVth ventricle. Naloxone, although successfully blocking morphine-induced respiratory depression does not appear to exert its effect on the blood-CSF barrier, but presumably either displaces the opiate from the receptor site or changes the agonist configuration of such receptors on brain stem respiratory neurons.

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948 ENKEPHALIN AND ENDORPHIN BREAKDOWN: STRUCTURE-ACTIVITY RELATIONSHIPS USING D-AMINO ACID ANALOGS.
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The analgesic potency of endorphin (LPH 61-91) has been attributed to its relatively slower breakdown as compared to shorter enkephalin (LPH 61-65) sequences (Jacquet and Marks, Science 194, 632, 1976). To provide direct evidence for this we compared breakdown of analogs stabilized against degradation with activity in vivo following intracisternal injection. Analogs used in this study were:

Tyr-D-Ala-Gly-Phe-Met	Tyr-D-Ala-Gly-Phe-Met.NH_2
Tyr-Gly-D-Ala-Phe-Met	Tyr-Gly-Gly-Phe-Met.NH_2
Tyr-Gly-Gly-D-Phe-Met	D-Ala^2-LPH 61-76 (α-endorphin)
Tyr-Gly-Gly-Phe-D-Met	D-Ala^2-LPH 61-77 (γ-endorphin)
	D-Ala^2-LPH 61-91 (β-endorphin)

Peptides were incubated with an ultrafiltrate of mouse brain and breakdown products separated on columns or polyamide sheets as previously described (Marks, Grynbaum and Neidle, BBRC 74, 1552, 1977).

Enkephalins: at shorter incubation brain aminopeptidases were blocked by D-Alanine in position 2 but not in position 3, or with D-Phe in position 4, and D-Met in position 5. Substitution in positions 3-5 blocked action by carboxypeptidases but not that of aminopeptidases. The presence of Met.NH_2 retarded C-terminal cleavage but in combination with D-Ala gave a peptide that was completely resistant to hydrolysis by brain enzymes. Injection of this analog 125 μg (100 g nonanesthetized rat) induced analgesia reversible by naloxone (2 mg/Kg) but with no overt behavioral changes. These data unequivocally show that biodegradation is an important factor in evaluating the activity of enkephalins in vivo since unsubstituted peptide was without marked effects.

Endorphins: insertion of D-Ala in position 2 of endorphins retarded the release of Tyr at short incubation periods but failed to block release of Tyr from β-endorphin at longer periods. For α- and γ-endorphins the pattern of release indicated action by carboxypeptidases at long (60 min) but not at short (5 min) incubation periods. In the case of β-endorphin the low yield of Gln and Gly indicated that the C-terminal is not readily susceptible to action by brain carboxypeptidases present in soluble extracts. D-Ala^2-endorphin when injected intracisternally in low doses gave a marked analgesic response accompanied by catatonia. Intraperitoneal injection of naloxone reversed these effects and resulted in wet-dog shaking behavior.

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949 AGE AND GENOTYPE DEPENDENT CHANGES IN MORPHINE INDUCED LOCOMOTOR ACTIVITY AND BRAIN HISTAMINE LEVELS. David E. McClain*, George N. Catravas and Herman Teitelbaum. Biochemistry Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Previous work (Lee and Fennessy, J. Clin. Exp. Pharm. Physiol. 3:179, 1976) has shown an inverse relationship between morphine induced changes in brain histamine levels and changes in locomotor activity in the same mouse strain (Commonwealth Serum Laboratories). We have been able to confirm this relationship using the C57BL/6J and DBA/2J strains of inbred mice approximately three months of age.

Morphine produces a dose dependent decrease in histamine levels in the C57BL/6J strain associated with a dose dependent increase in locomotor activity; in contrast, increased histamine levels and decreased locomotor activity are seen when the DBA/2J strain is given increasing doses of morphine.

When these experiments were replicated using six to seven month old mice the behavioral response to morphine was reproduced; whereas, the changes in brain histamine levels could not be replicated in the DBA/2J strain.

These findings suggest no causal relationship between morphine induced locomotor activity and brain histamine levels.

950 PHYSICOCHEMICAL CORRELATES OF ALCOHOL INTOXICATION. Michael J. McCreery* and Walter A. Hunt*. (SPON: J. Ribas). Neurobiol. and Behav. Sci. Depts., Armed Forces Radiobiol. Res. Inst., Bethesda, MD. 20014.

The precise way in which alcohols interact with membranes thus creating alterations in neuronal function remains unknown. Although the literature on the biological effects of alcohols is vast, most studies have dealt with only the anesthetizing properties of this broad class of depressants. Comparison of their actions as intoxicants in intact animals has remained unaddressed largely because of the absence of a suitable animal model. Previous studies of *in vitro* systems using a limited number of alcohols have suggested that their potency is directly related to their lipid/water partition coefficients (P). In the present effort, a broad range of structually divergent alcohols, diols, and other monofunctional alkanes were tested for their ability to intoxicate in whole animals. Male Sprague-Dawley rats were injected i.p. with several doses of a given drug and evaluated for the most severe signs of intoxication using behavioral endpoints described by Majchrowicz (Psychopharmacologia 43, 245 (1975)). From dose-response curves, the effective dose (ED) needed to produce ataxia 2 was determined. Of over 60 compounds tested, almost all induce a behavioral spectrum of intoxication virtually identical to that of ethanol. This includes amphiphilic compounds which are not alcohols such as propyl chloride and propanethiol. A plot of [log P] vs. [log ED] yields a straight line with a correlation coefficient equal to -0.9 indicating a high degree of predictability. The membrane concentration, the volume occupied within the membrane, and the thermodynamic activity have been successfully utilized by past workers to predict the anesthetic potency of a compound. In order to determine whether these physicochemical parameters could also be used as correlates of alcohol intoxication, partition coefficients were used to estimate the concentrations of each drug within the aqueous and nonaqueous regions of the animal. From these calculations each of these parameters was found to remain remarkably constant even though the ED varied over two orders of magnitude. Among the few exceptions were several alkanes which were ineffective in inducing intoxication even though their lipid solubility is very high. These data suggest that amphiphilicity of a compound is essential for inducing intoxication. Moreover, the three-dimensional structure of a compound is important in influencing its intoxicating efficacy only to the extent that its partition coefficient is affected.

951 CHANGES IN BRAIN TRYPTOPHAN AND TYROSINE FOLLOWING ACUTE AND CHRONIC MORPHINE. R.B. Messing, C. Flinchbaugh* and J.C. Waymire*. Dept. Psychobiol., Univ. Calif. Irvine, Irvine, CA 92717.

Morphine increases the turnover in brain of catecholamines and serotonin. Furthermore, synthesis of serotonin, and possibly of catecholamines, can be influenced by availability of their amino acid precursors, tryptophan and tyrosine. Therefore, we investigated the effects of morphine on these amino acids.

Morphine sulfate (5-20 mg/kg) increased brain concentrations of tryptophan and tyrosine 1-2 hr. after administration in a dose-dependent manner in male rats. Concentrations of these amino acids in blood serum decreased 30-45 min. post-injection, and then rose towards control values. The rise in brain amino acids could be antagonized by pretreatment with naloxone (1 mg/kg). Rats were made addicted to morphine by administration of 150 to 600 mg/kg of morphine sulfate in a slow release preparation in increasing doses, over a 10 day period. In these rats there was only a slight increase in brain tryptophan and no increase in tyrosine. Thirty min. after naloxone-precipitated withdrawal (0.5 mg/kg), tryptophan and tyrosine concentrations were increased in brain, in contrast to the decrease in these amino acids seen after naloxone is given to acutely morphinized rats.

The antagonistic effects of morphine and naloxone on brain tryptophan and tyrosine, and the differences in addicted and non-addicted rats, suggest that the changes in these amino acids in brain are due to specific effects of the drug.

These data support the hypothesis that increased amino acid precursor availability for serotonin and catecholamine biosynthesis may be involved in alterations of the metabolism of these neurotransmitters following morphine. (Supported by grant DA-01685.)

952 PERIAQUEDUCTAL GRAY AND THE MORPHINE ABSTINENCE SYNDROME IN THE RAT. James A. Mikula, Richard E. Wilcox, and Robert A. Levitt. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901

The periaqueductal gray (PAG) of the midbrain has been implicated in the analgesic action of narcotic drugs. It has also been found to contain a high concentration of opiate receptors and of endogenous morphine-like substances. The PAG may, therefore, be expected to play a major role in the abstinence syndrome which accompanies the chronic administration of narcotics. This syndrome results from drug withdrawal in an organism whose body has homeostatically adjusted to the presence of the narcotic. In the rat it includes hyperalgesia, climbing, jumping, "wet-dog" shakes, diarrhea, salivation and rhinorrhea. Since there is good evidence that the PAG is involved in narcotic analgesia we would expect the PAG to be involved at least in hyperalgesia.

There were 8 groups of 8 animals in the experiment. Four groups were first implanted with a cannula in the PAG. All animals then had a pellet implanted under the skin of the back. For half of the animals the pellet contained 75 mg of morphine while the other animals were implanted with a placebo pellet. 72 hours later withdrawal was precipitated by injecting naloxone either into the PAG (1 µg in 1.0 µl of water) or systemically (IP; 5 mg/kg) or by injecting saline either into the PAG or systemically. Pain sensitivity was assessed by means of the flinch-jump technique while the other signs of the abstinence syndrome were assessed by behavioral observation. All testing and histological analyses were done "blind" as to the animal's previous treatment.

Injection of naloxone, either into the PAG or systemically, produced hyperalgesia in morphine-dependent rats. In both cases the change in jump threshold was a reduction of about 30 percent. Systemic naloxone injections also produced a "full-blown" abstinence syndrome in morphine-dependent animals consisting of all of the common signs. None of these other signs were found in morphine-dependent animals injected with naloxone in the PAG. Neither hyperalgesia nor the other abstinence signs were produced by saline injections in morphine-dependent animals nor by naloxone or saline in placebo-implanted animals.

Similar amounts of hyperalgesia were produced by the PAG and IP naloxone injections, while the other abstinence signs were only produced by IP naloxone. Therefore, the PAG may be involved in the homeostatic adjustments responsible for hyperalgesia but not in the adjustments responsible for the other signs. These other adjustments may involve other CNS sites, and/or sites in the periphery. These results suggest the possibility of separating different effects of the narcotic drugs.

953 DIFFERENTIAL EFFECTS OF ETHANOL ON UNIT ACTIVITY IN CEREBELLUM AND OTHER BRAIN AREAS IN THE RAT. J. Mitra. Harrison Dept. Surgical Research, Division of Neurosurgery, Sch. Med., Univ. of Pennsylvania, PA 19104.

The effects of acute administration of ethanol on activities of single units were studied in rats. Units were recorded from the cerebellum (cortex and nuclei), the caudate n., Deiters n. and the reticular formation (RF). It was found that with a low dose of ethanol (100 mg/kg), units in the RF (rostral and caudal brain stem), the cerebellar nuclei and the caudate nucleus were activated while units in Deiters n., showed no appreciable change. With the same dose of ethanol, cerebellar Purkinje units showed a slight decrease followed by a transient increase in activity. A large dose (500 mg/kg) of ethanol initially depressed units in all structures under study. However, units in Deiters n. and in the cerebellar nuclei showed depression that was more prolonged than in other structures (cerebellar cortex, caudate n. and RF). Recovery from a large dose of ethanol was first evident in the RF (caudal followed by rostral). During recovery from the initial depression due to ethanol, units in Deiters n., the RF (rostral) and the cerebellar nuclei often showed periodic increases and decreases of unit discharge which lasted from 2-15 min. Our data suggest that a low dose (100 mg/kg) of ethanol activated units in the RF, the caudate n. and the cerebellar nuclei while a large dose (500 mg/kg) had a prolonged depressive effect on units in Deiters n. and the cerebellar nuclei. (Supported by P.H.S. Research Grant NIAAA 00902.).

954 EFFECT OF ALPHA-METHYL-P-TYROSINE ON ABSTINENCE INDUCED, SPONTANEOUS CONVULSIONS IN BARBITAL DEPENDENT RATS. William W. Morgan. Dept. Anat., Hlth. Sci. Cntr. at San Antonio, San Antonio, TX 78284.

Barbiturate dependence was produced in adult male Sprague-Dawley rats following the long term consumption of increasing concentrations of barbital in the drinking water. The highest concentration of barbital consumed was 4 mg/ml. Sodium saccharine was added to the drinking water to disguise the bitter taste of barbital. Equal concentrations of saccharine were provided to both control rats and barbital consuming rats. At the end of the drug regimen the barbital was abruptly withdrawn from the rats by the substitution of water which contained only saccharine. Just before barbital withdrawal and again at 16 hours after withdrawal, groups of control and barbital dependent rats were treated with 0, 30, 60, 125 or 250 mg/kg of alpha methyl-p-tyrosine (αMPT), intraperitoneally. All the animals were observed continuously during the first 48 hours following barbital withdrawal, and the incidence of spontaneous convulsions in each animal was recorded. Core temperatures were monitored with tele-thermometers at 12 hour intervals following barbital withdrawal. Only the highest dosage of αMPT produced hypothermia. After 48 hours of withdrawal the animals were sacrificed, and brains were collected for subsequent analysis of dopamine (DA) and noradrenaline (NA) concentration utilizing spectrofluorometric procedures. A dosage related decrease in spontaneous convulsions was observed in αMPT treated, barbital dependent rats. In a separate study these dosages of αMPT did not affect the incidence of convulsions induced by pentylenetetrazol administration or thyroparathyroidectomy-pinealectomy. All of the dosages of αMPT that were administered significantly decreased the synthesis of catecholamines as evidenced by a statistically significant decrease ($p<0.001$) in both DA and NA content. The cumulative data indicate that the effect of αMPT on convulsion incidence following withdrawal of barbital from dependent rats is related to the suppression of catecholamine synthesis. These results suggest that brain catecholamines may have a role in the expression of the barbital abstinence syndrome.

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955 INTRASEPTAL INJECTIONS: A PROCEDURE FOR STUDYING THE MODULATION OF THE CHOLINERGIC NEURONS PROJECTING TO THE HIPPOCAMPUS. F. Moroni*, S. E. Robinson*, D. L. Cheney and E. Costa. Lab. Preclin. Pharmacol., NIMH, Saint Elizabeths Hosp., Washington, D.C. 20032

Intraseptal injections of agonists and antagonists of two classes of putative neurotransmitters (opioid and catecholamine) have been performed in rats chronically implanted with cannulae. The acetylcholine (ACh) turnover rate (TR_{ACh}) has been monitored in the hippocampal formation and in other brain areas. The TR_{ACh} is calculated by measuring the incorporation of deuterium into ACh and choline mass-fragmentographically following the infusion of deuterated phosphorylcholine.

Morphine (35 and 70 nmoles) and β-endorphin (0.7 nmoles) injected directly into the septum fails to cause analgesia or electrical signs of convulsive activity but decreases the TR_{ACh} by approximately 50% in the hippocampus, leaving the ACh and Ch content unchanged. The TR_{ACh} in the cortex and striatum is not affected. The effect in hippocampus is antagonized by i.p. naltrexone (15 μmoles/kg). Furthermore, naltrexone (15 nmoles) directly injected into the septum, partially antagonizes the decrease of hippocampal TR_{ACh} induced by i.p. morphine (35 μmoles/kg). These data suggest that opiate agonists decrease the firing rate of cholinergic neurons projecting to the hippocampus.

On the contrary, intraperitoneal injections of amphetamine (27 μmoles/kg) increases the TR_{ACh} in the cortex, hippocampus and diencephalon by approximately 40%. Phenoxybenzamine (15 nmoles) injected into the septum curtails the increase of cortical TR_{ACh} elicited by amphetamine, but does not modify the increased TR_{ACh} in diencephalon.

Our data suggest that the α-adrenergic receptor agonists facilitate the ACh metabolism in septal cholinergic neurons and that opioid receptor agonists inhibit TR_{ACh} in these neurons. Intraseptal injections and measurement of TR_{ACh} is a useful pharmacological research tool to elucidate the structural organization of the septum.

956 EFFECT OF MORPHINE ON INTRACRANIAL SELF-STIMULATION IN RATS. William T. Nelson, Martin Brutus*, James E. Wilson, Jr.*, Robert A. Farrell*, Douglas R. Ocheret*, Steven J. Ellman and Solomon S. Steiner*. Dept. Psychology, The City College, New York, NY 10031.

The purpose of this study was to determine which sites in the brain yield an increase in intracranial self-stimulation (ICSS) response rates under morphine.

Twenty-five rats were subjects. Stimulation parameters and training procedures were similar to those of Bodnar et al. (Neuroscience Abstr., 1, 481, 1975). Each bipolar electrode was used in a monopolar fashion, allowing a comparison between each tip serving as cathode to a cortical screw anode.

After response rates stabilized, each animal received 7 days saline, 7 days morphine, 1 day morphine + naloxone, and 6 days post-drug saline. The first days of both pre-drug saline and morphine were discarded. Morphine doses were 2.5 and 5.0 mg/kg injected sc; naloxone dose was 1.0 mg/kg. ICSS rates were sampled 20 minutes post-injection.

Those electrode tips located in the locus coeruleus (LC) and those just lateral or ventral to it showed large facilitations under morphine. This area is delimited on either side by ICSS electrode tips which showed response depressions under morphine. Lateral hypothalamic (LH) placements yielded facilitations under morphine. Sites medial to the LH area, however, showed depressions under morphine. Substantia nigra (SN) placements were located throughout the zona compacta (SNC) and zona reticulata (SNR) and yielded facilitations and depressions in response rate which were not interpretable on an SNC/SNR distinction.

The facilitations occurred at the low C-T intervals only. The effect is an opiate-specific effect, in that naloxone reversed the observed morphine effect, whether facilitation or depression. The effect is not due to differences in individual animals' sensitivity to the drug, since there are animals which simultaneously showed a facilitation from one site and a depression from another site under the influence of morphine. There were two instances in which we obtained different drug effects from the two tips of the same electrode, thus indicating that each electrode tip is stimulating a discrete neural area. These results indicate that morphine exerts its reinforcing effect at very specific brain loci.

957 ANTAGONISM BY PROSTAGLANDIN (PG) OF MORPHINE-INDUCED EFFECTS IN THE PERIAQUEDUCTAL GRAY (PAG). G. A. Oltmans, J. E. Comaty*, and S. Ehrenpreis. Department of Pharmacology, Chicago Medical School, Chicago, IL 60612.

Ferri et al. (Psychopharmacologia 39:231, 1974) reported that PGE_1 injected intraventricularly reversed the analgesic effects of morphine. Ehrenpreis et al. (Nature [New Biol.] 245:280, 1973) have shown that PGs can antagonize narcotic action in vitro and postulated a common site of action for PGs and narcotics both in the periphery and CNS. We have now demonstrated that PGE can antagonize the analgesic and behavioral effects of morphine injected into the PAG, a site in the CNS directly implicated in opiate action (Jacquet and Lajtha, Science 182:490, 1973). Male albino Sprague-Dawley rats were stereotaxically implanted with a single chronically indwelling cannula aimed at one side of the PAG (AP=0.6; anterior L=0.75; V=6.5 below skull; Pellegrino and Cushman). Analgesia tests consisted of pinching both ears, pinching all paws, tail compression, and the hot plate. All measurements were quantitated, and each animal was given all four tests at various times following drug injections. In general, it was found that unilaterally administered morphine in PAG (in doses as low as 10 µg) produces a dose-dependent change from baseline in all tests except the tail-compression, which was all or none. At time of maximum analgesic response (20 µg morphine), infusion of as little as 2 µg PGE_2 or PGE_2-16,16 dimethyl ester in the same site immediately attenuated the morphine-induced analgesia. The PGs also rapidly reversed morphine-induced catatonia when it occurred. Effectiveness of the PGs was approximately equal to, and parallelled that of, an equimolar amount of naloxone infused into the PAG. These results support the previous reports that the PAG is a site of narcotic analgesia and behavioral effects and provides direct evidence for the involvement of PGE in opiate action in the CNS. (Supported in part by Biomedical Research Support Grant RR-05536, NIH, and by a grant from Hoffman-La Roche, Inc.)

958 EFFECT OF ETHANOL ON MEMORY STORAGE PROCESSES. Elizabeth S. Parker* and Ronald L. Alkana* (SPON: R. M. Julien). NIAAA, Rockville, MD 20857, School of Pharmacy, USC, Los Angeles, CA 90033, and Department of Psychobiology, UCI, Irvine, CA 92717.

It has been suggested that consolidation decrements underly the ethanol-induced deficits in memory storage that have been found in human and animal studies. Two preliminary experiments were conducted in which ethanol was administered pre- or post-training on a "step-through", one-trial, inhibitory avoidance task with mice. Retention was tested in the non-drug state in both experiments. In Experiment 1, immediate, post-trial, ip, ethanol (15% w/v) administration did not significantly affect retention at either 24 hours or 1 week after training. For example, median retention latencies were: Footshock (450 µA)-Saline (FS-S), 182 sec; Footshock-1.5 g/kg Ethanol (FS-1.5 g/kg E), 300 sec; FS-3.0 g/kg E, 300 sec; FS-4.5 g/kg E, 300 sec; and FS-6.0 g/kg E, 300 sec. Post-trial ethanol administration in the absence of footshock (NFS-E) did not significantly change latencies over NFS-S controls indicating that the ethanol injection was not aversive. In Experiment 2, 3 groups were added to examine the effects of pre-trial ethanol administration. In addition, the FS was lowered to 300 µA to try to reduce the ceiling effect seen in the retention latencies of the FS-E groups in Experiment 1. Injection of subanesthetic doses of ethanol (1.5 g/kg or 3.0 g/kg) 10 min pre-training produced a significant and virtually total amnesia compared to saline controls when retention was tested 1 week later. Even with the lowered footshock, however, there were no significant effects of post-trial ethanol administration. In summary, pre-trial ethanol administration impaired retention, whereas, immediate post-trial injections did not. These data do not support the consolidation hypothesis of ethanol's acute amnestic effects.

959 EFFECTS OF OPIATES AND OPIOID PEPTIDES ON RAT MOTILITY FOLLOWING INTRAVENTRICULAR AND INTRACEREBRAL INJECTIONS. Agu Pert, Jim Mitchell*, and Carlos Sivit*. Adult Psychiat. Br., NIMH, Bethesda, MD 20014.

Opiates have been found to exert complex effects on spontaneous motility in rodents. Low doses of morphine generally increase spontaneous activity while high doses have a biphasic action--an initial depression followed by hyperactivity. The purpose of the present studies was two fold: (1) to characterize and compare the effects of β-endorphin, $[D\text{-}Ala]^2$-met-enkephalin (D-Ala) and morphine on motility following intraventricular injections and (2) to localize the brain structures involved in mediating the effects of opiates on motility. Rats were implanted with either intraventricular or intracerebral cannalae guides. Following recovery they were injected with saline, varying doses of β-endorphin, D-Ala, morphine or naloxone and then tested in a photocell activity apparatus. Following intraventricular injections (25 µg), we found that all three opiate agonists produced an initial depression followed by excitation. The depressive phase lasted 1-2 hrs for D-Ala and approximately 3 hrs for morphine and β-endorphin. Tissue injections (5 µg bilateral) revealed differential effects of opiates on motility depending on the injection site. Injections into the nucleus accumbens produced only hyperactivity without a depressant component, while injections into the periaqueductal gray matter, surrounding tegmentum and pontine reticular formation produced only depression without excitation. Preliminary results indicate that injections into the thalamus, amygdala and caudate nucleus are relatively ineffective in modifying motility. Naloxone (10 µg bilateral) injected into the nucleus accumbens produced a transitory depression of activity. Systemic administration of naloxone (0.1 - 10 mg/kg) was also found to produce a dose-dependent depression of activity. These findings indicate that endogenous opiate peptides in brain may play a role in some aspects of motility. In addition it appears that the differential effects of opiates on activity are determined through different brain structures. Species and dosage differences in the action of opiates on motility may depend on the relative sensitivity of one system over the other.

960 CONSUMPTION OF A TRYPTOPHAN-DEFICIENT, CORN-BASED DIET ALTERS THE ANALGESIC DRUG POTENCY OF MORPHINE. Lee Phebus*, Philip Rowley*, Laurel Fisher*, and Loy D. Lytle. MIT, Dept. of Nutrition and Food Science, Cambridge, MA 02139.

We have previously shown that rats fed a tryptophan-deficient, corn-based diet (supplemented with normal amounts of vitamins, minerals, carbohydrates and fats) are hyper-reactive to presentations of noxious electric foot shock. The time course for the development of the electroshock hyperalgesia parallels that of the diet-induced depletion of brain tryptophan and serotonin, suggesting that brain serotoninergic neurons may play a role in nociception. The diet-induced hyperalgesia and reductions in brain serotonin can be reversed by: 1) offering animals previously fed the corn-based diet a casein based diet that contains adequate concentrations of tryptophan; 2) offering animals a corn-based diet that is supplemented with normal concentrations of tryptophan; or 3) injecting corn diet fed animals with various doses of tryptophan, or other drugs known to increase brain serotoninergic neurotransmission (Science 190: 692 (1975); Life Sciences 18: 707 (1976)).

In the present experiments, different groups of rats were fed either the tryptophan-deficient, corn-based diet or the nutritionally adequate casein control diet for 3 weeks; their "jump" responses to presentations of different intensities of electric foot shock were then determined following intraperitoneal injections of different doses of morphine sulfate (2.5, 5.0, or 10.0 mg/kg; salt weight) or the 0.9% saline vehicle (1 ml/kg).

	JUMP THRESHOLD (mA)	
Drug Treatment	Casein-Fed	Corn-Fed
Saline	0.79±.07	0.42±.06**
2.5 mg/kg Morphine	1.05±.16	0.50±.07*
5.0 mg/kg Morphine	1.46±.17*	0.60±.06*
10.0 mg/kg Morphine	1.33±.11**	0.59±.09

*All values are means ±S.E. (N=6). *p<.05; **p<.01 compared to casein-fed, saline injected group.*

Animals fed the casein control diet showed dose-related increases in the shock intensity necessary to elicit the jump response following morphine; in contrast, animals fed the corn-based diet did not show significant analgesia at any of the morphine test doses. Pharmacological or surgical reductions in brain serotoninergic neurotransmission also produce hyperalgesia and antagonize morphine analgesia, and suggest a role for these neurons in normal or opiate-induced alterations in nociception.

961 ANALGESIA FOLLOWING MICROINJECTION OF MORPHINE INTO THE NUCLEUS RAPHE MAGNUS OF ACUTELY DECEREBRATE RATS. Herbert K. Proudfit. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

Previous observations have shown that destruction of the n. raphe magnus (NRM) blocks morphine-induced analgesia (Proudfit and Anderson, Brain Research 98, 612, 1975) suggesting that the NRM is an essential part of the neuronal pathway mediating morphine's analgesic actions. However, it is unclear from these results whether morphine acts directly on the NRM or on some other part of the neuronal system. To distinguish between these possibilities, morphine was applied directly by microinjection into the NRM of acute decerebrate rats. Analgesia was tested using the tail-flick assay before and after microinjection of morphine in doses of 2.5-to-40.0 µg in 0.5 µl of saline. Within 1-to-9 minutes after morphine injection, the tail-flick response was maximally inhibited, and this effect was reversed by systemically administered naloxone (1.0 mg/kg, sc). In addition, analgesia induced by systemic administration of morphine (5.0 mg/kg, sc) was antagonized within 1-to-2 minutes by microinjection of naloxone into the NRM (0.4 µg/1.0 µl saline). Saline microinjected into the NRM (0.5 µl) had no effect on tail-flick latency. Also, microinjection of morphine in doses as high as 80 µg in a volume of 2 µl into areas of the brain stem rostral and dorsal to the NRM did not alter tail-flick latency. These results suggest that morphine acts directly on the NRM to produce inhibition of the tail-flick response to radiant heat. (Supported by USPHS Grant NS 12649.)

962 EFFECTS OF MET^5-ENKEPHALIN AND MORPHINE ON NEURONS OF THE TRIGEMINAL BRAINSTEM NUCLEAR COMPLEX. E. Puil, R.K. Andersen* and J.P. Lund. Dept. Pharmacology, University of British Columbia, Vancouver, and Centre de Recherches en Sciences Neurologiques, Université de Montréal, Montréal, Canada.

Since the analgesic effect of morphine is presently believed to be due to its interaction with opiate receptors in the neuraxis, it was of interest to compare the effects of this alkaloid with those of met^5-enkephalin, an endogenous opioid, on second order neurons activated by noxious inputs. There is considerable evidence that the subnuclei oralis (n. oralis) and caudalis (n. caudalis) of the spinal trigeminal complex are the major sites of reception for incoming nociceptive afferents. Extracellular recordings were made in these subnuclei of decerebrate cats, through one barrel of an assembly of glass micropipettes or with a tungsten microelectrode attached alongside. The remaining barrels of the micropipette were used for extracellular drug application by iontophoresis. Stimulation of the tooth pulp which has long been accepted to cause pain almost in pure form, was used as the main noxious input. The effects of the substances were also observed upon stimulation of the infra-orbital nerve (I.O.). The modality and size of the sensory receptive field which affected the particular cell were determined. Neurons which had a tactile receptive field but which did not appear to respond to "painful" inputs were generally unaffected by iontophoretically applied enkephalin. Others which received "painful" inputs were usually strongly inhibited by enkephalin. The inhibition was slow to develop but outlasted the period of application by as long as 20 minutes. These effects could be prevented by extracellular or intravenous administration of naloxone. The effects of morphine did not always resemble those of enkephalin; a depression of the amplitude of spikes was often observed upon application of morphine particularly in the n. oralis. Compared with enkephalin, morphine appeared to produce a weaker depression of neurons, whether firing spontaneously, activated by stimulation of tooth pulp or I.O. The inhibition by morphine was sometimes followed by an excitation lasting several minutes. These experiments suggest the presence of receptors for endogenous opioids in trigeminal neurons. Activation of such receptors appears to be responsible for the observed depression by enkephalin of cells responding to noxious stimulation. The data also indicate that the mechanism of action of morphine may not be identical to that of enkephalin on these cells. Alternatively the analgesic effects of morphine on facial and oral structures may not be mediated through direct activation of these receptors within the trigeminal spinal nucleus.
Supported by Medical Research Council of Canada.

963 THE ROLE OF CATECHOLAMINES IN SELF-INJECTION OF COCAINE AND APOMORPHINE. David C. S. Roberts*, Michael E. Corcoran, Ronald M. Clavier, and Hans C. Fibiger. Div. Neurol. Sci., Univ. of British Columbia, Vancouver, Canada V6T 1W5.

There is evidence to suggest that self-administration of amphetamine and cocaine is reinforced by the central catecholaminergic agonist properties of these drugs. However, there is little known concerning the relative importance of specific catecholaminergic fiber systems in this behavior. The present experiments were designed to evaluate the role of two major noradrenergic (NA) and dopaminergic (DA) projections in intravenous self-injection of cocaine and of apomorphine, a direct DA agonist. Stereotaxic infusions of 6-hydroxydopamine (6-OHDA) were used to destroy either the DA innervation of the n. accumbens or the NA projections innervating the hypothalamus, hippocampus, and cortex.

Twenty rats were used, each fitted with a chronic intravenous cannula. During daily 4-hr sessions, each depression of a lever produced an intravenous injection of 0.20 ml of drug. Cocaine self-injection was maintained at a dosage of 0.75 mg/kg/inj. Some rats were also allowed to self-inject apomorphine at a dose of 0.06 mg/kg/inj.

Lesions of the dorsal and ventral NA bundles that reduced hippocampal and cortical NA by 96% and hypothalamic NA by 80% failed to have any effect on self-injection of cocaine. In contrast, 6-OHDA-induced lesions of the n. accumbens significantly altered the intake of cocaine. This effect was dependent upon the degree of DA depletion in the accumbens: In the five rats in which the greatest depletion of DA was achieved (90%), intake of cocaine was reduced to 20 to 40% of prelesion levels. Typically these rats would respond at a low rate, and only in the first 1 to 2 hrs of the session. The same animals showed no change in rate or pattern of self-injection of apomorphine after the lesion. Identical lesions of the n. accumbens had only transient effects upon food-reinforced operant responding, suggesting that the prolonged disruption of cocaine self-injection was not the result of motor deficits. The results suggest that DA terminals in the n. accumbens may mediate some of the positive-reinforcing properties of cocaine.

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964 EFFECTS OF BARBITURATE ON SPINAL CORD INHIBITORY PATHWAYS. Howard C. Rosenberg and Michiko Okamoto. Cornell Univ. Med. Coll. New York, N. Y. 10021.

The segmental reflex system of the lower spinal cord has long been an important model system for studying drug effects on the CNS. Barbiturates are important since other depressant drugs are often compared to barbiturates in order to ascertain any selectivity that such drugs might possess. Pentobarbital effects on 3 types of spinal cord inhibitory pathways have been studied. Cats were anesthetized with halothane and a tracheotomy, C1 section and carotid artery ligation were performed. Respiration on room air was then begun. The spinal cord was exposed by laminectomy from L4 to S1 and a hindleg was dissected to allow stimulation of specific nerves. Reflex activity was recorded from appropriate ventral roots that had been sectioned near their exits from the spinal column.

In agreement with published reports, pentobarbital enhanced presynaptic inhibition. Doses required to do so were about the same as needed to depress the unconditioned 2N response.

Postsynaptic (direct) inhibition has been reported not to be specifically affected by pentobarbital (Weakly et al., Arch. Int. Pharmacodyn. 171, 1968). Effects of pentobarbital doses from subthreshold to those that almost abolished the 2N response were studied by adjusting the strength of the test stimulus in many steps, and then by stepwise infusion of many doses of pentobarbital in the same animal. Decreased inhibition could be demonstrated over a wide dose range in contract to the apparent changes that are only secondary to decreased size of the unconditioned 2N response (decreased synaptic drive).

Recurrent (Renshaw cell-mediated) inhibition has been reported to be prolonged by barbiturates, although no consistent change in the maximum degree of inhibition was stated (Wilson and Talbot, J. Gen Physiol. 502, 1960). No specific effect of pentobarbital on the magnitude of recurrent inhibition was noted. There was an apparent increase which was secondary to decreased synaptic drive throughout the range of doses from threshold to those causing almost total loss of the 2N response. In most experiments there was a prolongation of this inhibition.

Thus, acutely administered barbiturate increases presynaptic, decreases postsynaptic and prolongs recurrent inhibition. These data are of interest to compare with those obtained during barbiturate withdrawal showing recurrent and presynaptic, but not postsynaptic, inhibition to be decreased (Rosenberg and Okamoto, Fed. Proc. 35, 1976). (Supported by ADAMHA grant #DA-00591).

965 COMPARISON OF MORPHINE ACTION ON SENSORY INPUT RECORDED IN FIVE BRAIN SITES. J. Salamy S. Sands and N. Dafny
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Certain brain structures including the central gray (CG), mesencephalic reticular formation (RF), nucleus parafasicularis (PF), and caudate nucleus (CN) have been implicated in morphines effect on pain, analgesia, tolerance, and motor disturbances respectively. Those structures were therefore selected for the present study. Specifically, the influence of morphine upon these sites was determined by its ability to modify the averaged evoked response to visual stimulation. The lateral geniculate body (Lgb) was employed as a sensory control site. Permanent electrodes (60 μ diameter) were implanted several days prior to experimentation in 11 Sprague-Dawley rats (200-300g). After 1 hour adaptation to the recording cage 4 consecutive pre-drug control averages consisting of 32 visual evoked responses (VERs) each, were obtained at 10 minute intervals. Similarly, 4 sets of VERs were obtained following the successive administration of morphine in each of 1, 5, 10 and 30 mg/kg i.p. doses and one dose of naloxone (1 mg/kg i.p.). The incremental doses of morphine followed by naloxone were injected at one hour intervals. The first average of each set was initiated 15 minutes following injection. Subsequent averages were obtained at 10 minute intervals. VER modification was regarded as an alteration in the amplitude of one or more of the P_2, N_2, or P_3 components of $\pm$ 2 standard errors from control values for each animal. The Lgb responded in a dose related fashion with increasing dosages modifying more and more responses. The CG appeared most sensitive to the influence of morphine. The VERs were maximally altered at the lowest dose (1 mg/kg) with little further change associated with higher doses. The responses of the RF and PF displayed comparable sensitivity. Only a few VERs were modified at the lowest dose (1 mg/kg) and the maximum effect was attained at 10 mg/kg. The CN exhibited the least responsiveness to morphine. However, the direction of response changes (increases or decreases of the various components) were dose related. Naloxone reversed the effect of morphine in all structures studied. These results indicate that the effects obtained were a specific result of morphine and that morphine influenced these structures differentially. Supported by USPHS Grant No. DA 00803.

966 IMMUNOHISTOCHEMICAL LOCALIZATION OF ENKEPHALINS IN RAT BRAIN. Madhabananda Sar*, Walter E. Stumpf, Richard J. Miller*, Kwen Jen Chang*, and Pedro Cuatrecasas*. Depts. Anat. and Pharma., Sch. Med., UNC-CH, Chapel Hill, N.C. 27514, and Burroughs-Wellcome Co., Research Triangle Park, Durham, N.C. 27709.

The distribution of enkephalins in rat brain was investigated by immunohistochemical staining technique using antisera to leucine-enkephalin and methionine-enkephalin. Adult male Sprague Dawley rats were perfused through the aorta with 3% paraformaldehyde and 0.5% gluteraldehyde in sodium phosphate buffer. Brains were removed. Forebrain, midbrain, pons and medulla oblongata were dissected out, dehydrated through alcohol and xylene, and embedded in paraffin. 4 μm sections were cut, deparaffinized and stained immunohistochemically by a modified immunoperoxidase bridge technique.

Immunoreactive staining for enkephalin (leu or met) was observed in nerve fibers and terminals in many regions of the brain. The localization was similar when antisera to met or leu-enkephalin were used. The localization is specific for enkephalins since antisera to leu or met enkephalin previously absorbed either with leu or met enkephalin fail to produce a positive staining in brain sections. The regions of localization include in the forebrain: lateral septum, amygdala, hippocampus, globus pallidus, nucleus caudatus, and nucleus accumbens; in the midbrain: nucleus interpeduncularis and periaqueductal gray; and in the lower brain stem: nucleus parabrachialis, nuclei raphes, locus ceruleus, nucleus tractus solitarii and formatio reticularis. The results of the histochemical staining reveal that certain structures which stain positively for enkephalin, closely correspond to the distribution of opiate receptors in the brain.

967 PUPILLARY CHANGES FOLLOWING MICROINJECTIONS OF OPIOIDS, SYMPATHOMIMETICS AND CHOLINOMIMETICS INTO THE OCULOMOTOR NUCLEUS IN THE DOG. Lawrence G. Sharpe, Wallace B. Pickworth* and William R. Martin. NIDA, Addiction Research Center, Lexington, KY 40511

Male and female beagle-type dogs had indwelling guide cannulae (19 ga) implanted with the tips located above the oculomotor nuclear complex (OMN). All animals were acclimated to sling restraints and during experimentation a concentric chemitrode (30 ga inner, 23 ga outer, insulated except at the tips) was lowered into a site where electrical stimulation (20 Hz, 0.5 msec duration, 2-6V) produced marked miosis without ocular movements. Drugs dissolved in sterile saline were then injected in a 0.5 μl volume over 1 min into this site. Pupil diameter was measured photographically. Morphine (5 μg), normorphine (5 μg), WIN 35,197-2 (0.5-2 μg), but not SKF 10,047 (5 μg), produced miosis lasting 45 min or longer. The rapidly metabolized sympathetic amines, dopamine (10-20 μg), norepinephrine (5 μg) and epinephrine (5 μg) produced transient miosis, whereas the longer acting α-adrenergic agonists, clonidine (5 μg) and methoxamine (5 μg) produced a prolonged miosis. Acetylcholine plus physostigmine (5 μg each) and carbachol (0.5 μg) produced marked mydriasis whereas atropine (0.5 μg) produced miosis. In another study in which decerebrate dogs were used, carbachol (10-20 μg) also caused mydriasis when microinjected into the OMN. These data suggest that there is a cholinergic inhibitory and possibly a catecholaminergic facilitatory mechanism modulating the visceral nuclei of the OMN.

968 DEMONSTRATION OF STEREOSPECIFIC OPIATE BINDING IN CULTURES OF FETAL MOUSE DORSAL ROOT GANGLIA AND SPINAL CORD. Eric J. Simon*, Jacob M. Hiller*, Stanley M. Crain and Edith R. Peterson* (SPON: C.S. Raine). Dept. Med., New York Univ. Coll. Med., N.Y. 10016; Dept. Neurosci., Albert Einstein Coll. Med., Bronx, N.Y. 10461.

Sensory-evoked synaptic networks in the dorsal horn regions of fetal mouse spinal cord cross-sectional explants with attached dorsal root ganglia (DRGs) can be selectively depressed by exposure to analgesic concentrations of morphine and other opiates, and these effects are reversed by naloxone (Crain et al., this vol.). Correlative analyses have been made of the opiate receptor binding levels in these cord-DRG cultures as well as in cultures of isolated DRGs and deafferented cord explants. Profuse neuritic outgrowths developed, primarily due to NGF-stimulated DRGs, extending for several mms beyond the explant zone on a collagen gel substrate (which thinly coated the 22 mm culture coverslips). Prior to some of the assays, the explants (ca. 1 mm diam.) were extirpated for separate analyses of the explants and the neuritic outgrowth zones. The latter were collected together with the entire remaining collagen substrate after removal of the explants. All assays were made on pooled groups of 8 cultures.

Homogenates of these explants (1-3 weeks in vitro), were found to exhibit stereospecific binding of the potent opiate antagonist, diprenorphine. Four times as much ^{3}H-diprenorphine was bound by homogenates of isolated DRG cultures (2500 cpm/mg protein) than by deafferented spinal cord cultures (700 cpm/mg). (All protein determinations were corrected for collagen substrate.) Similar cultures of non-neuronal explants exhibited no stereospecific binding of opiates. It was further found that the neuritic outgrowth zones of DRG cultures had four times the binding of the DRG explants (i.e. 2000 cpm/mg protein vs. 600 cpm/mg). On the other hand, the binding per mg protein in explants of spinal cord extirpated together with their attached DRGs was more than twice that of entire cultures of deafferented cord, and the binding by the neuritic outgrowths of these co-cultures was less than that of isolated DRG outgrowths. This shift in opiate receptor localization may be due to the growth of many receptor-bearing DRG neuritic processes into the spinal cord explants, consonant with bioelectric and histologic evidence of NGF-enhanced DRG-innervation of dorsal cord in these cultures (Crain and Peterson, Brain Res. 79:145,'74).

This report presents the first demonstration of opiate binding on neurites of dorsal root ganglion cells and the first direct evidence of presynaptic binding of opiates.

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969 MORPHINE EFFECTS ON PROTEIN SYNTHESIS IN NORMAL AND REGENERATING FACIAL NUCLEUS NEURONS. Raymond S. Sinatra* and Donald H. Ford. Dept. Anat. Cell Biol., Downstate Medical Center SUNY, Brooklyn, N.Y. 11203

In addition to specific interactions at regional binding sites opiates induce numerous secondary alterations in CNS biochemistry including pronounced inhibition of neuronal RNA and protein synthesis. Axotomy has been associated with an altered metabolic state and the effects of morphine on the increased synthetic drive of axotomized neurons has not been previously reported.

Acute morphine effects on the accumulation of ^{3}H-l-lysine was studied by LM radioautography in normal and regenerating facial neurons. At predetermined intervals post axotomy, radioactivity present in various compartments within regenerating and normal neurons from adult male Wistar rats was compared with similar cells from rats receiving 30mg/kg morphine sulfate I.V. At 14 and 21 days post axotomy, regenerating neurons were larger and their grain count/unit area increased significantly when compared with unoperated controls. In unaxotomized neurons, the accumulation of lysine into the nucleus and nucleolus was decreased significantly 60 min after morphine administration. In regenerating neurons, morphine inhibition of lysine incorporation appeared even more pronounced. Nuclear lysine uptake was significantly depressed at 3 and 7 days, while maximal inhibition of cytoplasmic incorporation occurred at 14 days post axotomy. Morphine administration decreased nucleolar lysine incorporation at all time intervals observed.

Electron microscopic examination of normal and regenerating neurons suggested that chronic morphine administration induces ultrastructural alterations in organelles associated with RNA and protein synthesis. In nonoperated cells and regenerating cells 3 days post axotomy, shrinkage of RER cisternae with a con-comitant aggregation of polysomes within the intercisternal cytoplasmic matrix was observed. The characteristic nucleolar enlargement observed in the control regenerating cells did not occur after morphine treatment.

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970 CONSEQUENCES OF MORPHINE PELLET IMPLANTATION IN NEONATAL FEMALE RATS. Theo Sonderegger, Sue Overing* and Emery Zimmermann. Dept. Anat. and Brain Res. Inst., Sch. Med., UCLA, Los Angeles, CA 90024.

Previous studies of effects of neonatal exposure of rats to morphine have employed daily injections of the narcotic to produce addiction. Since repeated handling early in life may confound drug effects upon neuroendocrine and behavioral responses, the present study investigated long-lasting consequences of early morphine exposure utilizing a modified pellet-implantation procedure developed by us to minimize handling of the pups (Proc. Soc. Exp. Biol. & Med. 154: 435-438, 1977).

Charles Rivers CD strain albino rats with 8 pups each were used in a split litter design. On Day 1 of life female pups were weighed and assigned to a treatment group. On Day 5 or Day 11 pups were anesthetized briefly with ether and implanted subcutaneously with a morphine (M) or a lactose placebo (P) or no pellet (NP). Pups were not further handled until they were weighed, weaned and separated into treatment groups on Day 21.

Surviving pups totaled 59 (Day 5) and 47 (Day 11); overall mortality was 15%. Body weights of M-treated groups were depressed below those of P- or NP-groups through Day 63. Open-field testing on Days 28-30 did not differentiate between treatment groups. After being put on 23-hr food deprivation and tested in the Lashley III maze at approximately 72 days of age, the M-treated animals made significantly more errors and took longer to complete the daily trials ($p<0.01$).

At Day 90, littermates were bred to the same male and all animals became pregnant. Litter sizes, birth weights and sex ratios of the offspring did not differ between groups. These offspring are being studied further.

At 150 days of age Day 5 animals were anesthetized with ether and a blood sample was quickly taken from the jugular vein and the plasma was assayed fluorometrically for corticosterone. Resting levels of corticosterone for the M, P and NP groups did not differ significantly. On Day 215, animals were subjected to a 3-min ether stress and blood samples taken 30 min later. The M group exhibited a smaller ($p<0.05$) steroid rise in response to ether than did the other groups.

These results are comparable to those obtained previously using injection techniques and further indicate that neonatal exposure to M produces long-lasting growth, behavioral, and neuroendocrine deficits.

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971 DIRECT, INTRACEREBRAL SELF-ADMINISTRATION OF OPIATES IN THE RAT. Elliot A. Stein and James Olds.[1] Div. of Biology, Calif. Inst. of Technology, Pasadena, CA 90025.

It has long been realized that opiate agents possess powerful reinforcing properties. This has been readily demonstrated by animals which rapidly learn to press a lever to obtain an intravenous injection of morphine (Weeks, J.R., Science 138, 143 [1962]). However, little is known of the central loci or mechanisms by which these drugs produce their hedonic effect.

In an attempt to determine which areas within the central nervous system subserve the reinforcing properties of opiates, a series of experiments have been designed to allow rats to self-administer drugs into discrete subcortical nuclei. Male, Holtzmann rats are surgically prepared with 26 g guide cannulas aimed stereotaxically to lie just dorsal to the area of interest. Sites chosen include those known to support electrical self-stimulation as well as those implicated as sites of morphine action from binding studies. During experimental sessions, a 31 g drug cannula is lowered into the chosen area and rats are placed into a rectangular plexiglass box with a pedal at each end. One pedal is arbitrarily chosen as the active one with each press resulting in a single injection (0.1 µg) of drug (volume per injection = 10 nl). The opposite pedal is inactive and serves as a control for changes in general activation. Experiments are run over a twenty hour period with retests performed three days later.

Results indicate that only those structures which contain both high opiate receptor density as well as those which support electrical self-stimulation will support opiate self-administration. These include anterior lateral hypothalamus, preoptic region, septum, and lateral hypothalamus. Inactive regions include lateral thalamus, caudate nucleus and central gray. Locus coeruleus sites have resulted in equivocal responding, although motor seizures have been seen in two of these animals from injections in this area.

These data indicate that there may be specific central structures subserving opiate hedonic actions which appear to overlap areas known to be rewarding to electrical stimulation. Results are discussed as significant factors in repeated addict relapse phenomena. (Supported by NIH Postdoctoral Fellowship DA05030 and Grant DA 01541 from NIDA)

[1]Based on data collected prior to Dr. J. Olds' death.

972 METHIONINE ENKEPHALIN QUANTIFICATION: A SPECIFIC RADIOIMMUNOASSAY. Sue Sullivan*, Huda Akil*, Stanley Watson and Jack D. Barchas. Nancy Pritzker Lab. Behav. Neurochem., Dept. Psych. Behav. Sci., Stanford Univ. Sch. Med., Stanford, CA 94305.

An antibody directed against methionine enkephalin has been raised in rabbits. The antigen used was met-enkephalin coupled to BSA using a glutaraldehyde reaction. The antibody has been used for an immunoassay and for immunohistochemical localization of met-enkephalin in rat brain. The following substances were found not to cross react in the immunoassay at concentrations of 1 µM: Gly-Gly-Phe, Gly-Phe, Gly-Gly, Tyr-Gly, Tyr-Gly-Gly, Phe-Leu, β-endorphin, β-lipotropin, α-endorphin, leu-enkephalin, naloxone, levorphanol, morphine, and the individual amino acids of met-enkephalin. The assay detects from 0.5 to 100 pmoles of met-enkephalin. Using this RIA, levels of met-enkephalin in normal human CSF were found to be 3.1 ± 1 pmoles/ml, which correlated well with enkephalin levels measured in the opiate receptor assay. Supported in part by DA 01207 and DA 01522.

973 A PARADOXICAL "AVERSIVE" PROPERTY OF LEUCINE-ENKEPHALIN: CONDITIONED TASTE AVERSION IN RATS. L. Switzman*, L. Hammer*, P. Shizgal and Z. Amit (SPON: Z. Amit) Centre for Research on Drug Dependence, Psychology Department, Concordia Univ., Montreal, Canada.

Belluzzi and Stein (Nature, in press) have demonstrated intraventricular enkephalin self-administration in rats. Morphine is also self-administered intraventricularly (Amit et al., Psychopharmacology, 1976, 48, 291-294) and, paradoxically, it can induce a conditioned taste aversion (CTA) to a saccharine-water solution (Switzman et al., in prep.). We investigated the ability of leucine-enkephalin (100, 200 or 300μg), methionine-enkephalin (100, 200 or 300μg) and d-alanine-enkephalinamide (25, 75 or 148μg) to induce a CTA in rats. All animals were placed on partial fluid deprivation for seven days such that water was presented for 2 successive ten-minute periods each day. On the eighth day, a saccharine-water solution was presented for ten minutes followed one minute later by an infusion of either of the enkephalins, the vehicle control solution (Ringer's) or a pH control solution. The rats were maintained on the partial deprivation schedule for the subsequent five days and on the sixth day, saccharine solution was again presented for ten minutes (test day). Fluid intake was recorded on all days. Only the leucine-enkephalin (200μg) group significantly decreased saccharine intake on the test day (83.13% of baseline compared to an increase of 109.89% for the Ringer's controls, $t=2.446$, $df=18$, $p < .05$), thus demonstrating a leucine-enkephalin induced CTA to saccharine flavored water. These results are interesting in light of the report by Belluzzi and Stein (ibid) in which leucine-enkephalin was self-administered at a higher rate than that of methionine-enkephalin. It is worthwhile to note that only the middle dose of leucine-enkephalin induced a CTA. This parallels the inverted U-shaped dose response relationship which we have observed for CTAs induced by both systemic and intraventricular morphine injections.

974 ETORPHINE-INDUCED ANALGESIA AND CATATONIA IN THE RAT. Beverly E. Thorn, Robert A. Levitt and Lisa M. Ohotzke*. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901.

Etorphine hydrochloride is a fast-acting narcotic analgesic, several thousand times more potent than morphine. The increase in potency of etorphine compared to morphine is accounted for both by increased blood-brain barrier affinity and by increased receptor binding. We studied the analgesic and catatonic properties of etorphine both when administered systemically and when microinjected into the periaqueductal gray (PAG) region of the midbrain.

The flinch-jump technique was used to assess pain sensitivity (five series of ascending and descending shocks administered for 1.0 sec every 30 secs.), and the bar test to study catatonia (the forepaws are placed across a bar 4 inches off the floor). Increases in jump thresholds indicate analgesia, while increases in time on the bar indicates catatonia.

Each group consisted of 8 rats. Systemic doses of etorphine were 5,10,50, or 100 μg/kg. PAG doses were 1.0 or 3.0 μg. Each animal was used only once in one experiment and was administered etorphine only one time. The animals received a flinch-jump test followed by a bar test (taking 10 min.). They were then injected with drug (either water or etorphine, either IP (0.25-0.35 ml.) or into the PAG (1.0 μl)). While systemic Ss received only one injection (water or one of the doses of etorphine), PAG animals received both water and one dose of etorphine, presented in a counterbalanced order and separated by a four-day interval.

Rats injected with water or 5 μg/kg etorphine IP were neither analgesic nor catatonic. 10, 50 and 100 μg/kg IP etorphine produced a progressively greater analgesia, with a progressively greater catatonia also being produced by the 50 and 100 μg/kg doses. The jump threshold increase and the catatonia test results correlated .85. PAG injections of 1.0 μg etorphine produced both analgesia and catatonia in half the animals, while 3.0 μg etorphine produced both analgesia and catatonia in all the animals. Mean jump threshold scores increased from .132 ma to .329 ma for the 1 μg group, and to .503 ma for the 3 μg group. With PAG injections the jump threshold increase and catatonia test results correlated .77.

These studies show that the effective doses for producing analgesia and catatonia in the PAG are much lower than the effective systemic doses. Moreover, the high correlations between degree of analgesia and presence of catatonia for both systemic and PAG injections may suggest a common mechanism and substrate for these two actions of the narcotic drugs.

975 POSSIBLE RELATIONS BETWEEN BRAIN OPIATES AND SOCIAL BEHAVIORS. Tom Vilberg*, Noel Bean*, Paul Bishop*, Ken Porada* and Jaak Panksepp. Dept. Psych., BGSU, Bowling Green, OH 43403.

The functions of endogenous opiate systems in the brain are yet to be conclusively identified, however the increasing number of endogenous morphine mimetics and the widespread distribution of opiate binding sites in the brain suggests that more than the modulation of pain is involved.

Possible similarities between the dynamics of narcotic addiction and the formation of social bonds in animals (i.e. speed of development, strength and persistence) has led us to test the hypothesis that one function of these systems is the elaboration of social behaviors. Since acute morphine often has debilitating effects, we employed the receptor blockade strategy to test this possibility. Effects of naloxone were tested in opiate naive animals on diverse measures of discrete social behaviors in several species.

Naloxone severely disrupted pup retrieval in mice. The mother would repeatedly pick up pups and drop them without returning them to the nest. Infant (11 day old) mice showed higher levels of separation-induced activity (55.3% increase) following naloxone (1 mg/kg) than saline treatment. Naloxone-induced activity increases were not seen in adults. In 1 day old chickens, naloxone (5 mg/kg) increased latency to sleep induced by simulated maternal contact by 108%. Tonic immobility, a response linked to social fear, was decreased by 10.2% in duration in juvenile chickens. Three to five day old chicks increase the frequency of their distress vocalizations in response to social separation by 585% when treated with naloxone (2.5 mg/kg) as compared to control conditions. Adult cats were found to vocalize 52% more when given naloxone (1 mg/kg). All these effects were statistically reliable and usually visually obvious.

These findings stand in contrast to the difficulty which has previously been encountered in demonstrating any behavioral effects of naloxone in opiate naive animals. So far naloxone has had reliable effects on every innate social behavior we have analyzed, suggesting that endogenous opiate systems may modulate emotional or motivational functions underlying social behavior in many species. In general it appears that naloxone exacerbates the distress that results from social isolation and reduces the comforting effects of social contacts. Accordingly, these data are consistent with the possibility that endogenous opiate systems mediate social reward processes in the CNS. However, since we have presently only tested innate behaviors, the results are also consistent with the possibility that endogenous opiate systems merely modulate the expression of species-typical behaviors.

976 IMMUNOCYTOCHEMICAL LOCALIZATION OF OPIATE PEPTIDES AND β-LIPOTROPIN IN RAT BRAIN. Stanley J. Watson, Huda Akil* and Jack D. Barchas. Nancy Pritzker Lab. Behav. Neurochem., Dept. Psych. Behav. Sci., Stanford Univ. Sch. Med., Stanford, CA 94305.

With the discovery of the opiate peptides in mammalian brain, a number of questions have arisen concerning their physiology, biochemistry and anatomy. The substance β-lipotropin (β-LPH) has been proposed as a precursor peptide for methionine-enkephalin and several of the endorphins. Using specific antisera directed against these substances (β-LPH antisera obtained from Dr. C. H. Li--Hormone Res. Lab., UCSF), we have carried our immunohistochemical localization studies in rat brain. Generally the opiate peptides (e.g., enkephalins) and β-LPH are distributed variably throughout the brainstem. Relatively little of any of these substances can be seen in cortex, cerebellum, or hippocampus. High concentrations of both the enkephalins and β-LPH are seen in hypothalamus, amgydala, periaqueductal central grey, and paraventricular nucleus of the thalamus. Certain areas, such as median eminence and the arcuate nucleus, contain more β-LPH and much less enkephalins, whereas areas such as the lateral septal nucleus and certain cranial nerve nuclei contain more of the enkephalins and very little β-LPH. The existence of β-LPH in specific neurons in brain lends weight to its hypothesized role as an opiate peptide precursor. Supported in part by DA 01207 and DA 01522.

977 CHRONOTOXICITY OF BARBITURATES AND CHLORAL HYDRATE IN MICE. *Ulysses G. Whitworth, *Alvin Sermons and Charles A. Walker, School of Pharmacy, Florida A and M University, Tallahassee, FL 32307.

Twenty-four hour LD_{50} values of secobarbital, pentobarbital, phenobarbital, hexobarbital, and chloral hydrate were determined in Swiss Webster mice. Three experimental groups of animals were adapted for three weeks in an environmental room equipped with an automatically-timed light cycle lasting from 0800 to 2000 hours daily. For group A, injections for toxicity analysis were made intraperitoneally at D-0600, L-1200, L-1800 and D-2400. Group B were injected with hexobarbital and pentobarbital every three hours of the 24 hour cycle, (D-0600, L-0900, L-1200, L-1500, L-1800, D-2100, D-2400 and D-0300). Group were injected every three hours before the injection. Peak toxicity has reached at 0600 with all drugs studied except chloral hydrate which was 180 degrees out of phase. The drugs were least toxic at 1200 hours at a time when the animals were sleep, with the exception of chloral hydrate which was least toxic at 0600 hours. The greatest percent change in toxicity occurred during the hours of 1800 and 1200. Chloral hydrate changes may be explained by a different mechanism of biotransformation. This investigation probably indicates the existence of a circadian and food dependent biological rhythm with sedative-hypnotic drugs. (Supported by a Grant from the Office of Naval Research).

978 BRAIN SITES OF NALOXONE REVERSAL OF MORPHINE CATATONIA IN THE RAT. Richard E. Wilcox and Robert A. Levitt. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901.

Analgesic doses of morphine in rats produce sedation. Higher doses result in more profound decreases in spontaneous activity culminating in a catatonic-like state. Previous work in our lab suggested a correlation between brain sites having many opiate receptors and the ability of naloxone to reverse morphine-catatonia when administered via chemical brain stimulation (CBS).

Subjects were 24 Long-Evans male rats weighing between 250 and 400 grams at the start of the experiment. Each rat was implanted with one 23 gauge guide shift aimed at head of the caudate nucleus (HC; n=8), periaqueductal gray (PG; n=8), or cerebellar white matter (CB; n=8). Drugs used for CBS were naloxone hydrochloride (10 µg) dissolved in sterile isotonic saline and isotonic saline. Previous work in our lab has demonstrated that the ED50 for systemic naloxone reversal of catatonia induced by 80 mg/kg morphine is 400 µg/kg. All CBS were administered in 1 µl of solution. Subjects were used as their own controls, receiving naloxone or saline in counterbalanced order on two test days separated by one week.

Behavioral testing was as follows. Animals were stimulated with 80 mg/kg morphine sulfate i.p. Thirty minutes later animals were tested for catatonia with a bar test. Animals keeping both forepaws on the bar for 30 seconds were given a CBS with naloxone or saline. At the time of testing the experimenters were not aware of the identify of the solutions used for CBS. Ten minutes later the bar test was repeated. On the second test day drug conditions for CBS were reversed. Thus, half of the animals in each brain site received naloxone the first time they were run and half received saline. Thus each animal received both naloxone and saline in the same brain site. Histological verifications were done without knowledge of the behavioral results.

No reversals occurred following saline stimulation in any of the 24 animals. Naloxone in the PG produced 5 reversals (out of 8 animals; 10 seconds or less on the bar after naloxone) while naloxone in the HC produced 4 reversals (out of 8 animals). Zero out of 8 animals responded to naloxone stimulation in the CB. A chi-square test comparing the number of reversals and failures to reverse at the sites of brain stimulation revealed significant differences at the $p<.025$ level.

Catatonia reversal is the first behavioral effect of morphine found to be mediated by the HC. Reversal of catatonia in HC and PG but not in CB supports the contention that high concentrations of opiate receptors are required for this action. This suggests that morphine-catatonia is a direct effect of the narcotic on opiate receptors in the brain.

979 ETHANOL PREFERENCE IN GENETICALLY SELECTED HYPERTENSIVE AND HYPOTENSIVE MICE. W. Gibson Wood, Merrill F. Elias* and Clyde A. Pentz*. Dept. Psych. Syracuse Univ., Syracuse, N.Y. 13210.

Mice selected for hypertension and hypotension were tested for ethanol preference using 8 different concentrations (Experiment 1). Hypertensive mice showed a greater preference for ethanol than hypotensive mice at 7 of the 8 ethanol concentrations used. Subsequent ethanol preference testing (Experiments 2 & 3) using hypertensive and hypotensive mice from a segregating F_2 generation, derived from the crossing of hypertensive and hypotensive hybrids ($F_1 \times F_1$) demonstrated that the greater preference shown by the hypertensive mice in Experiment 1 was not related to blood pressure phenotype in a casual manner (e.g., pleiotropic or linked genes). Results of the three experiments emphasized the importance of genetic tests designed to determine whether relationships between blood pressure phenotype and ethanol preference do, in fact, reflect casual relationships or mere artifacts of selection.

980 NALOXONE REVERSAL OF MORPHINE ANALGESIA: AN ANATOMICAL ANALYSIS. Randall D. Young, Robert A Levitt, and Maxine Weyant*. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901.

One reliable procedure for measuring the response to pain in the rat is the jump-flinch technique. Using this procedure, it is possible to obtain a baseline measure of pain sensitivity, a measure of morphine analgesia, and a measure of naloxone reversal of morphine analgesia; all in the same animal and during the same testing session.

Adult rats served as subjects. Each animal was implanted with a 23g cannula aimed at the periaqueductal grey (PAG) of the midbrain. On the test day each subject was given a baseline measure of jump-flinch responding then injected with 10mg/kg morphine-sulfate. After a 30 min. period for drug onset the animal was retested for pain sensitivity and the animal was injected with either 1.0µg or 0.1µg of naloxone in the PAG (in 1µl water) or 1µl distilled water. Following a 10 min. period the animal was retested for pain sensitivity.

Each jump-flinch measurement was composed of 5 trials of 1 sec foot shock delivered in an ascending and descending series at 30 sec intervals.

The ascending series was continued until a "jump" response was elicited, then the series was reversed until the foot shock failed to elicit a response. A jump response is a highly agitated motor response consisting of two or more paws being off the grid floor simultaneously. The five shock intensities eliciting jump responses were averaged to give the jump threshold.

Morphine injections were effective in raising jump thresholds by about 100%. 1µg naloxone was effective in returning jump thresholds to baseline while 0.1µg returned the jump thresholds only half-way back to baseline. Distilled water was ineffective in reversing morphine analgesia. These results were confirmed statistically.

This paradigm appears promising for investigating brain areas mediating morphine analgesia. Such investigations have already been started in this lab, focusing on the caudate nucleus, amygdala, and hippocampus; brain areas shown to be high, medium, and low in opiate-receptor density.

981 ABNORMALITIES IN THE UNTREATED OFFSPRING OF FEMALE RATS TREATED NEONATALLY WITH MORPHINE. E. Zimmermann, Theo Sonderegger and Sue Overing*. Dept. of Anat. and Brain Res. Inst., Sch. of Med., UCLA, Los Angeles, CA 90024.

Previous studies demonstrated long-lasting growth, behavioral and neuroendocrine effects of neonatal exposure to morphine (M) in female rats (Zimmermann et al, Life Sci. 20: 639, 1977). Friedler recently called attention to diminished size of the offspring of rats treated with M prior to conception (Fed. Proc. 36: 1001, 1977). To investigate possible cross-generational effects of neonatal M treatment, Sprague-Dawley female rat pups (Charles Rivers) were implanted with a M, placebo (P) or no pellet (NP) on Day 5 or Day 11 of postnatal life using a split-litter design. On Day 90 these animals were bred with the same sire for each treatment block. A total of 319 pups were produced. Litter sizes (4 to 17), sex ratios and birth weights were similar for the M, P and NP groups. Excluding the 5 pups described below, pups of M-treated mothers weighed less than controls on Day 28. Female offspring of Day 5 M-treated mothers showed unaltered sensitivity to M when tested on the hot plate on Day 67. On Day 92 female pups of Day 11 M-treated mothers showed decreased sensitivity to the analgesic action of M (10 mg/kg s.c.) but exhibited unaltered sensitivity to the hot plate prior to M injection. Offspring of both Day 5 and Day 11 M-treated mothers exhibited diminished ($p<0.05$) plasma corticosterone elevations 30 min after acute injection of M (30 mg/kg) on Day 138.

Five female pups from 3 litters manifested a striking syndrome of growth, sensory and motor deficits. They had significantly lower body weights and lacked peripheral pain sensation and fine motor control. The hindlimbs were normally held in full extension and locomotion was achieved by trunk movements with awkward forelimb assistance. They showed both diminished resting and ether stress-induced levels of corticosterone, but evidenced normal vaginal cyclicity. When challenged with naloxone (5 mg/kg) these animals, unlike controls, showed no elevation of corticosterone levels.

These findings are consistent with those of Friedler and indicate that exposure of female rats to M early in life may produce M tolerance as well as serious growth, neurological and endocrinological deficits in their progeny. The basis for such effects is not known, but may involve a drug action on the immature germ cell which becomes evident after fertilization and development. (Supported in part by USPDHS grant DA826 and NIH RR-07055).

NEUROCHEMISTRY

982 CHARACTERIZATION OF RNA SYNTHESIS WITH RABBIT AND GERBIL BRAIN SLICES IN VITRO. J. Albrecht* and T. Yanagihara, Dept. of Neurol., Mayo Clinic and Mayo Med. School, Rochester, MN. 55901

In order to characterize the newly synthesized RNA, rabbit and gerbil brain slices were incubated with [^{3}H] uridine and radio-labeled RNA was investigated with nuclear, rough endoplasmic reticulum membrane and free polyribosomal fractions. TCA-insoluble specific radioactivity was highest in the nuclear fraction. The specific radioactivity of the membrane fraction was twice as much as the free polyribosomal fraction. Actinomycin D (up to 0.1 μg./ml. incubation medium) inhibited [^{3}H] uridine incorporation to similar extent (up to 40%) in these subcellular fractions, while the higher concentration of Actinomycin D resulted in more selective inhibition of the free polyribosomal fraction. The results were similar in rabbit and gerbil. Polyribosomal RNA was then extracted with phenol and further fractionated with oligo (dT) cellulose chromatography. The results indicated that 52% of labelling of rabbit and 26% of gerbil RNA were associated with poly(A)-containing (messenger) RNA. In contrast to the cell culture system or the in vivo system reported in the literature, low concentration of Actinomycin D (up to 0.08 μg./ml.) did not increase the relative labelling of poly (A+) RNA. There was even relative decrease of poly (A+) RNA with rabbit brain. Polyribosomal RNA was further characterized with continuous sucrose density gradient (15 to 30%) ultracentrifugation. In both rabbit and gerbil system, the major radio-labelling occurred in non-ribosomal RNA even with the fractionation of poly (A-) RNA. The poly (A+) RNA showed the major peak just ahead of 18S RNA while the large portion of poly (A-) RNA sedimented faster than 28S RNA. Though the messenger activity of poly (A-) RNA remains to be established, both poly (A+) RNA and poly (A-) non-ribosomal RNA were vulnerable to Actinomycin D. The present results demonstrated that RNA synthesis with brain slice in vitro favors synthesis and/or nucleocytoplasmic transport of messenger RNA, and that this system provides a convenient tool for studying the fate of messenger RNA under various physiological and pathological conditions. (Supported by the grant NS-06663 from NIH)

983 SPECIFIC (^{3}H)OUABAIN BINDING TO DIFFERENT AREAS OF BRAIN AND SOME PERIPHERAL ORGANS OF CAT: EFFECT OF CHRONIC ETHANOL TREATMENT. Shailesh P. Banerjee and Virendra K. Sharma*. Department of Pharmacology & Toxicology, University of Rochester, School of Medicine & Dentistry, Rochester, New York 14642.

Chronic ethanol treatment of cats, increased specific (^{3}H)-ouabain binding by 63% in cerebral cortex, 47% in cerebellum, 84% in amygdala and 100% in hippocampus when the binding assays were performed in the presence of 160 nM (^{3}H)ouabain. There was no significant change in specific (^{3}H)ouabain binding in hypothalamus, thalamus, corpus striatum and brain stem following chronic ethanol ingestion. Specific (^{3}H)ouabain binding to microsomal fraction derived from ethanol-treated cat heart, liver, spleen and kidney increased by 70%, 75%, 63% and 42% respectively as compared to that found with microsomal fractions obtained from control animals. Scatchard analysis revealed that enhancement of specific (^{3}H)ouabain binding following chronic ethanol treatment in several peripheral organs and some areas of cat brain is primarily due to changes in densities of ouabain binding sites. Since ouabain is a specific inhibitor of ($Na^+ + K^+$)-ATPase the present observations suggest that the molecular mechanism for the enhancement of ($Na^+ + K^+$)-ATPase activity after chronic ethanol ingestion may be due to increased net rate of synthesis of ($Na^+ + K^+$)-ATPase molecules or exposure of non-functional enzyme system following conformational change of plasma membrane. (Supported by the American Heart Association and H.L. 18185.)

984 COMPARISON OF IN VIVO AND IN VITRO PHOSPHORYLATION OF SYNAPTIC MEMBRANES. Robert F. Berman*, John P. Hullihan* and John E. Wilson. Department of Biochemistry, University of North Carolina, Chapel Hill, North Carolina 27514.

In vivo and in vitro phosphorylation patterns of synaptic membrane proteins were compared. In vivo phosphorylation was carried out by injecting 1 mCi of ^{32}P-orthophosphate into the ventricles of adult rats via chronically implanted cannulas and sacrificing after 40 minutes. Brains, minus brainstem, cerebellum and olfactory lobes, were homogenized and synaptosomal fractions were isolated by ultracentrifugation of the P_2 pellet on a continuous sodium diatrizoate gradient. The synaptosomes were osmotically disrupted, and the resulting ^{32}P-labeled synaptic membranes were further fractionated by SDS-polyacrylamide (7.5%) slab-gel electrophoresis. Unlabeled synaptic membranes from non-injected rats were phosphorylated in vitro by incubation with [γ^{32}P]ATP (4μM) for 2 minutes prior to SDS-polyacrylamide electrophoresis. Following electrophoresis, the gels were stained (Coomassie blue) and the resultant protein patterns were scanned by densitometry. The protein patterns for in vivo and in vitro labeled synaptic membranes were identical. In contrast, autoradiograms of these gels demonstrated striking differences in the phosphorylation patterns between in vivo and in vitro labeled synaptic membranes. While similarities were apparent, several protein bands which were labeled by ^{32}P by the in vivo phosphorylation procedure, did not appear to be labeled when phosphorylation was carried out in vitro. Cyclic-AMP (10μM) was found to maximally stimulate the in vitro phosphate labeling of two protein bands which were not labeled by the in vivo procedure. These results suggest the possibility that the regulation of phosphorylation of synaptic membrane proteins differs between the in vivo and in vitro states. (Supported in part by NIH Grant NS-07457 and by The Alfred P. Sloan Foundation).

985 TWO FORMS OF RAT CEREBRAL MICROSOMAL ($Na^+ + K^+$)-ADENOSINETRIPHOSPHATASE WITH DIFFERING SUSCEPTIBILITY TO PERIODATE INHIBITION John M. Bertoni* and George J. Siegel. Dept. Neurology, Sch. Med., Univ. of Mich., Ann Arbor, Mich. 48109.

50% inhibition of rat cerebral ($Na^+ + K^+$)-adenosinetriphosphatase [($Na^+ + K^+$)-ATPase] and K^+-paranitrophenylphosphatase (K^+-pNPPase) activities occurred reproducibly after 30 minutes of preincubation of NaI-extracted microsomes (0.1 mg protein/ml) in 25 μM periodic acid and 150 mM imidazole HCl (pH 7.4) at 23°C. Inhibition of Mg^{++}-ATPase or Mg^{++}-pNPPase activities under the same conditions was only 20% and 8%, respectively. ($Na^+ + K^+$)-ATPase activity was inhibited by 40% after 1 minute of exposure to periodate under these conditions. Thereafter, the inhibition progressed more slowly, reaching 60% after 80 minutes preincubation. Microsomes were preincubated with various concentrations of periodate for 30 minutes under these conditions. Inhibition of ($Na^+ + K^+$)-ATPase activity reached 40% at 10 μM periodate and thereafter progressed to 50% and 70% at 25 μM and 100 μM periodate, respectively. A similar periodate concentration response curve was obtained for inhibition of K^+-pNPPase activity under the same conditions. At least 95% inhibition of ($Na^+ + K^+$)-ATPase activity was produced by ouabain indicating that the measured activity represents the cation transport enzyme. Increasing the microsomal protein concentration in the exposure medium reduced the inhibition produced by 25 μM periodic acid. Inhibition of K^+-pNPPase activity could not be reversed by two washes of the periodate treated microsomes. To explore the possibility that a soluble oxidation product was inhibitory, microsomes were preincubated with 50 μM solutions of formic acid, formaldehyde, acetaldehyde, and glyceraldehyde and no inhibition was found under conditions in which 25 μM periodate produced 40% inhibition. These findings indicate that the cation transport enzyme in rat cerebral microsomes exhibits at least two forms distinguished by their sensitivity to periodate. The relative protection of half the enzyme activity may be related to enzyme conformation or orientation within the microsomal membrane. The ($Na^+ + K^+$)-ATPase and K^+-pNPPase activities show the same biphasic response suggesting that the same periodate-sensitive group(s) may be involved.

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WITHDRAWN BY AUTHOR

987 REGULATION OF PHENYLETHANOLAMINE N-METHYLTRANSFERASE SYNTHESIS AND DEGRADATION. Roland D. Ciaranello. Dept. Psychiatry, Stanford University Medical Center, Stanford, CA 94305

Adrenal medullary phenylethanolamine N-methyltransferase (PNMT) is under dual control by impulses from the splanchnic neurons, and by glucocorticoids from the adrenal cortex. Several lines of evidence suggest that these regulators operate by different biochemical mechanisms. Administration of reserpine and 6-hydroxydopamine, drugs which cause a reflex activation of the splanchnic nerves causes a marked increase in adrenal PNMT activity in both normal and hypophysectomized rats. In contrast, dexamethasone or ACTH have no effect on PNMT in normal rats, although both drugs will restore to normal the profound decrease in PNMT levels brought about by hypophysectomy.

After hypophysectomy PNMT levels fall markedly; this is associated with a decrease in immunotitratable PNMT molecules. Dexamethasone partially restores PNMT activity and immunotitratable enzyme levels, suggesting that the in vitro measurement of enzyme activity reflects the number of PNMT molecules present. Dual radioactive amino acid labelling studies show that the fall in PNMT levels after hypophysectomy is the result of accelerated degradation of the enzyme. PNMT turnover studies in dexamethasone-treated hypophysectomized rats show that dexamethasone restores PNMT levels by inhibiting the accelerated degradation caused by hypophysectomy. Dexamethasone has no apparent effect on the rate of PNMT synthesis.

Studies on the kinetic properties of PNMT from hypophysectomized and normal rats fail to disclose differences in affinity of PNMT for its substrates. Stability of PNMT at 50° is profoundly accelerated after hypophysectomy, however, suggesting an in vitro as well as in vivo susceptibility of the enzyme to denaturation after hypophysectomy. Thermal stability of PNMT appears to depend on the presence of an endogenous stabilizing factor which binds to the enzyme, protecting it against heat denaturation. This factor is present in control rats, is lost after hypophysectomy, and can be restored by dexamethasone treatment. Thus this endogenous factor may act in vivo to stabilize PNMT against intracellular proteolysis as well as acting in vitro to protect the enzyme against thermal denaturation. Intracellular PNMT proteolysis may, then, be regulated by this endogenous substance.

988 ACTIONS OF PHENYTOIN ON AChE SYNTHESIS IN CULTURED CHICK EMBRYO MUSCLE TREATED WITH PARAOXON. C. Michael Cisson*. Richard K. Entrikin, and Barry W. Wilson. Univ. of Calif., Davis, CA 95616.

The combination of phenytoin (diphenylhydantoin, DPH) and exercise alleviated functional disabilities of dystrophic chicks and reduced characteristically high muscle AChE activities (Entrikin et al., Science 195:873, 1977). In addition, 15 µg/ml DPH reduced the total AChE production of chick embryo muscle cultures by 34% and AChE released into the medium by 40%. Cellular AChE levels and net protein synthesis were not altered (Cisson et al., Fed. Proc., 36:498, 1977). These studies suggested DPH decreased net synthesis of AChE activity. To further study this question, we briefly treated cells with 3×10^{-8}M paraoxon (which inhibited 90% of the AChE activity) and then measured synthesis of new AChE in a normal medium or in one containing 15 µg/ml DPH. During the first 2 hours of recovery, cells treated with paraoxon + DPH produced significantly less AChE activity and maximal recovery was delayed by 30 minutes compared to cells treated with paraoxon alone (Table 1). Eight hours after treatment, net synthesis of AChE activity (Δcell + Δmedium) by cells treated with paraoxon + DPH was also significantly less compared to cells that were treated with paraoxon alone. DPH did not affect general cell protein synthesis. The results show that DPH decreases AChE activity in cultured chick embryo muscle by reducing net synthesis of AChE activity. (Supported by NIH grants ES 00202, AM 16716, and NS 05308, and the MDA).

Table 1

Percentage of Paraoxon-treated Cells

Expt.	Mean Cell AChE 0-2 hours*	Total AChE 0-8 hours***	Total Protein 0-8 hours**
1	88.0±6.4 (P<0.01)	74.2±4.0 (P<0.001)	101.8±6.0 (N.S.)
2	80.5±8.5 (P<0.005)	87.3±8.6 (P<0.01)	100.0±7.8 (N.S.)

*N = 5 samples in Expt. 1 and 6 samples in Expt. 2 of 3 dishes each sample time.

**N = 6 dishes in each experiment.

989 DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN RETINA: SUBCELLULAR DISTRIBUTION. Yvonne Clement-Cormier* and Dianna Redburn (SPON: G.A. Robison). Dept. of Neurobiology and Anatomy and Dept. of Pharmacology, Univ. Tex. Med. Sch., Houston, Tx. 77025.

Biochemical and pharmacological analysis of subcellular fractions from rabbit retina demonstrated the presence of an adenylate cyclase activity which is selectively stimulated by low concentrations of dopamine. The highest specific activity of adenylate cyclase was found in the P_2 fraction which is enriched in synaptosomes from amacrine and perhaps to a lesser extent from bipolar and horizontal cells. Adenylate cyclase activity was also observed in the P_1 fraction which contains synaptosomes from photoreceptor cells; however, adenylate cyclase sensitivity to dopamine was not associated with this fraction. A half maximal increase in the activity of the enzyme in the P_2 fraction occured in the presence of 4×10^{-6} M dopamine. A high degree of pharmacological specificity was demonstrated for the receptor-cyclase complex. Chloropromazine, a dopamine antagonist, competitively inhibited the activity of the enzyme in this fraction with a calculated inhibition constant of 5×10^{-8} M. A study of the relative effects of the cis-trans isometric forms of the thioxanthene, flupenthixol showed that the -isomer of flupenthixol was a more potent dopamine antagonist of adenylate cyclase activity in the retina than the -isomer. In addition, the (+) isomer of butaclamol was more potent than the (-) isomer in blocking the stimulation of adenylate cyclase by dopamine. The inhibition constant for (+) butaclamol was calculated to be 4.5×10^{-8} M. Dopamine agonists, apomorphine, N-methyldopamine (epinine) and 2 amino-6,7-dihydroxy-1,2,3,4-tetrahydronapthlene (ADTN) mimicked the action of dopamine on adenylate cyclase activity in retinal homogenates and subcellular fraction P_2. Dopamine has been previously localized by histofluoresence within the amacrine cells of the retina. In addition, we have demonstrated the localization of ^{3}H-dopamine uptake and release systems specifically within the amacrine (P_2) synaptosomal fraction. These data suggest that amacrine cells may stimulate adenylate cyclase systems within bipolar and/or ganglion cells by interacting with dopamine receptors which are pharmacologically similar to those previously characterized in the rat striatum. (Supported in part by a grant from the Pharmacuetical Manufacturer's Association.)

990 THE EFFECTS OF LEAD INTOXICATION ON THE BRAIN UPTAKE OF CALCIUM IN ADULT ALBINO RABBITS by S.L. Cookson*, J.D. Mann*, C.S. Kim*, J.T. Gatzy* and L.A. O'Tuama, (SPON: Troy A. Reaves, Jr.,) Depts of Neurology, Medicine and Pharmacology, Univ. North Carolina, Sch. Med., Chapel Hill, N.C. 27514

A technique for rapidly assessing the passage of solutes across the blood-brain barrier during a single circulatory pass has been successfully developed and extensively used (Oldendorf, Brain Research, 24:372-376, 1970). In the present study, this method has been applied to investigate the brain uptake of calcium in lead poisoned rabbits. Lead intoxication was induced in 30 day old albino rabbits by the oral administration of lead carbonate, 165mg/day for 5 days. Controls consisted of age matched non-lead exposed rabbits. Experimental procedures were carried out under ether anesthesia at the end of the lead exposure period. ^{45}Ca and [^{3}H] water were made up in buffered Ringers lactate to a total volume of 0.4cc and rapidly injected as a single bolus into the left carotid artery through a 27 ga. needle. Final calcium concentration in the injectate was 1.531 mM of which 0.031 mM was ^{45}Ca. Ten seconds after injection, animals were sacrificed by decapitation. This time for sacrifice was chosen on the basis of ^{14}C-inulin indicator dilution studies which show that transit time through rabbit brain for an intravascular marker is 8 seconds or less. In each experiment, the brain was quickly removed and the left hemisphere divided into cortical gray and deep white matter for determination of ^{45}Ca and [^{3}H] water by liquid scintillation spectroscopy. A portion of the right hemisphere was analyzed for lead content by atomic absorbtion spectroscopy. A brain uptake index (BUI) (Oldendorf, Loc. cit.) was calculated for left hemisphere samples. Final values were corrected for residual blood volume. Brain lead content was found to be 0.035μg/gram and 0.377 ± 0.10μg/gram for control and lead intoxicated groups respectively. The BUI for cortical gray matter was 0.98 ± 0.06 (SEM)% for controls and 0.72 ± 0.04% for lead poisoned animals ($p<0.01$); for deep white matter the values were 1.45 ± 0.10 (SEM)% and 1.37 ± 0.09% for controls and experimentals (NS). Other studies have suggested that a primary effect of lead intoxication is disruption of transport systems associated with the neural barriers (Lorenzo, A.V., Gewirtz, M.:Proc Am Soc Neurochemistry, 231, 1977; Kim et al, Environ Health Perspectives, in press, 1977). The finding in this study of a 24% decrease in calcium uptake in cortical gray matter suggests that lead intoxication may also disrupt systems for transport of calcium at the blood-brain barrier.

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991 EFFECTS OF LIPID INTERMEDIATES, ALIPHATIC ALCOHOLS AND DETERGENTS ON SYNAPTOSOMAL LYSOPHOSPHOGLYCERIDE : ACYL-CoA TRANSFERASE ACTIVITY. D. R. Corbin*, R. MacQuarrie*, and G. Y. Sun. Sinclair Comp. Med. Res. Farm, Univ. of MO, Columbia, MO 65201.

The enzymic transfer of arachidonyl groups to 1-acyl-glycerophosphorylinositol (GPI) and 1-acyl-glycerophosphorylcholine (GPC) has been shown to occur in the synaptosomal fraction of mouse brain. This enzyme has been implicated as being important in regulating the turnover of polyunsaturated acyl groups of membrane phosphoglycerides, especially those present in the synaptic vesicles and synaptic plasma membranes. This membrane-bound enzyme was found to be greatly inhibited by Triton X-100 (0.01% or higher), a non-ionic detergent. On the other hand, sodium taurocholate, an ionic detergent, gave slight stimulation at the 0.01% level but exerted an inhibitory effect at higher concentrations (0.1% or higher). Excess bovine serum albumin (BSA) added to the incubation system also inhibited the transfer of (1-^{14}C)-arachidonate to the lyso acceptor molecules. This effect may be due to an excessive binding of the fatty acid substrate to BSA. A number of lipid intermediates with a free hydroxyl group, such as 1-acyl-phosphatidic acids, diacylglycerols and monoacyl glycerols were found to exert an inhibitory effect (25%) to arachidonyl transfer to 1-acyl-GPI and GPC. Furthermore, arachidonyl groups were not appreciably transferred to these lipid intermediates, further indicating the specificity of the substrates for acyl transfer. Ethanol at low concentrations (0.5-1.0%) gave slight stimulation to the acyl transfer system but became inhibitory at higher concentrations. Aliphatic alcohols with increasing chain length gave increasing inhibition to the enzymic system, a phenomena consistent with the fact that membrane-bound enzymes are greatly affected by changes in the hydrophobic regions of the membranes. (Supported in part by USPHS Research Grant NS12906 and NSF BNS76-24338).

992 RECIPROCAL MODULATION OF AMINO ACID AND SUGAR UPTAKE BY NOREPINEPHRINE AND EXTRACELLULAR K+ IN ASTROCYTES IN VITRO. C. J. Cummins, O. Z. Sellinger, and R. A. Glover (SPON: S. Winans). The University of Michigan, Ann Arbor, Mich. 48109.

We (CJC and RAG) presented details of a new technique for the cultivation of rat brain astrocytes at the last Neuroscience Meeting. By morphological, cytochemical, histochemical and biochemical criteria, this technique produces highly purified populations of astrocytes.

It is generally known that extracellular concentrations of K+ affect the membrane potential, oxygen uptake and ATP levels in preparations of astrocytes in vivo and in vitro. Uptake into confluent cultures of astrocytes of 2-deoxy-d-glucose (2-DOG) and methionine was observed in a buffer composed of 5.3 mM KCl, 150 mM NaCl, 1.0 mM $CaCl_2$, 0.6 mM $MgCl_2$, 1.6 mM KH_2PO_4, 4.3 mM Na_2HPO_4, at pH 7.2. To test the effects of elevations in extracellular K+ concentrations, K+ was replaced isoosmotically by Na+. At 11.9 mM K+, the uptake of 2-DOG was increased over control levels (at 6.9 mM K+). Increases in K+ above 11.9 mM were without effect. Norepinephrine (NE) and serotonin (5-HT) at 1.0×10^{-6} M decreased 2-DOG uptake. GABA diminished uptake, but not significantly. These effects do not appear secondary to alterations in cAMP levels or intracellular ionic perturbations, since dibutyryl cyclic AMP and ouabain were without effect.

Methionine was transported linearly for 150 seconds up to 2.4×10^{-6} M, yielding distribution ratios (met_{in}/met_{out}) of approximately 2.0. K+ concentrations of 11.9 mM decreased uptake of methionine at most methionine concentrations. NE at 50×10^{-9} M increased the uptake 2-fold while ouabain and dibutyryl cAMP had opposite effects.

These observations indicate that extracellular K+ concentrations and NE may act as reciprocal regulators of amino acid and hexose uptake in vitro, and they may suggest that in vivo K+ and NE regulate the levels of extracellular metabolites in brain by modulation of astrocytic uptake.

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993 POTENTIATION OF STIMULUS-SECRETION AND STIMULUS RESPIRATION IN ISOLATED CORTICAL TISSUE BY CYCLIC AMP (cAMP) AND ISOPROTERANOL Joseph T. Cummins and Elizabeth Keller, V.A. Hospital, Sepulveda, CA 91343, and Dept. Med. Pharmacol. Therup., Univ. California, Irvine.

Rat brain cortical slices were maintained in Krebs-Ringer bicarbonate and depolarized by electrical pulses (100Hz, 10 V). Stimulus-respiration was measured by changes in the steady-state level of reduced pyridine nucleotides [NAD(P)H] after electrical pulses. Stimulus-secretion was measured by following the rate of endogenous glutamate release before and after the application of electrical pulses. Isoproteranol (10^{-5}M), cAMP (10^{-3}M), and the phosphodiesterase inhibitor RO 20-1724 (10^{-5}M) potentiated by 75% of the increase in NAD(P)H due to electrical pulses. The potentiation by cAMP is inhibited by the β-adrenergic blocker propranolol (10^{-5}M), but not the α-adrenergic blocker phenoxybenzamine (10^{-5}M). In parallel experiments on superfused cortical slices cAMP potentiated by the 80% release of endogenous glutamate by electrical pulses. Thus cAMP potentiated stimulus-respiration and stimulus-secretion to the same extent. These observations imply that brain respiration and secretion is directly controlled by components of a β-adrenergic-adenylate cyclase system. These biochemical reactions of stimulus-respiration and stimulus-secretion may be a measurement of a potentiated state in which adrenergic neurones exert functional control over brain respiration and neurosecretion.

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994 ENZYMIC DEGRADATION OF DIACYL-GLYCEROPHOSPHORYLINOSITOLS BY BRAIN SUBCELLULAR MEMBRANES. O. C. Der* and G. Y. Sun. Sinclair Comp. Med. Res. Farm, Univ. of MO, Columbia, MO 65201.

The implications of important functional roles of the acidic phospholipid, diacyl-glycerophosphorylinositols (GPI), in metabolism of excitable membranes has prompted us to study the biochemical characteristics of enzymes degrading this type of molecule in brain. The substrate, 1-acyl-2-^{14}C-arachidonyl-GPI, was prepared by enzymic transfer of 1-^{14}C-arachidonate to 1-acyl-GPI which in turn was obtained by degradation of diacyl-GPI by phospholipase A_2. Incubation of the labeled diacyl-GPI (app. 40,000 cpm, 5 nmole) was performed in a system containing 0.5-1 mg of membrane proteins (0.32M sucrose with 50mM Tris, pH 7.4) and sodium taurocholate and deoxycholate (1 mg each) in a total volume of 0.5 ml. Approximately 25-30% of the labeled substrate were hydrolyzed in 1 hr at 37°C. Hydrolysis was reduced 60% when incubated without detergents. Maximal hydrolysis was observed in the presence of both detergents but Triton X-100 (0.1%) gave nearly complete inhibition. Enzymic degradation proceeded linearly up to 1 hr of reaction time and the products formed (mainly diacylglycerols) were proportioned to the externally added substrate as well as the amount of enzymic protein (up to 2 mg). The enzyme was specific for degradation of diacyl-GPI since 1-acyl-2-^{14}C-arachidonyl-GPC (choline) could not serve as an active substrate. Under the present incubation condition, very little degradation of diacyl-GPI by phospholipases A_1 or A_2 was found. Further assay of the enzyme with various purified subcellular and subsynaptic fractions indicated that activity of the enzymes was 1 1/2 times higher in the microsomal than in the synaptosomal fractions. Very little activity was found in the myelin. Upon subfractionation of the synaptosomal fraction, the hydrolase activity seemed to reside mainly in the synaptic vesicles and synaptic plasma membranes with little or no activity in the mitochondria fraction. (Supported by USPHS Research grant NS12960).

995 MEASUREMENT OF NEWLY SYNTHETIZED ^{3}H-3-METHOXYTYRAMINE AS INDEX OF NERVE IMPULSE FLOW DEPENDENT DOPAMINE RELEASE IN RAT STRIATUM. A.M.DiGiulio*, A.Groppetti*, F. Cattaben*, A.Maggi* and S.Algeri* (SPON: T. C. Westfall) Inst. of Pharmacology; Inst. of Pharmacology and Pharmacognosy, Univ. of Milan and M.Negri Inst. for Pharmacological Research, Milan, Italy.

It has been proposed that changes of 3-Methoxytyramine (3-MT) concentrations give a reliable index for Dopamine (DA) release into the synaptic cleft. In fact drugs known to reduce neuronal DA activity (i.e. γ-Butyrolactone and Apomorphine) produce a decrease of striatal 3-MT concentrations.

However, administration of d-Amphetamine (AMPH)(1mg/kg i.v.), a drug also reported to inhibit firing rate of DA cell bodies in Substantia Nigra, results in an increase of striatal 3-MT. This suggests that AMPH may release DA by mechanisms not dependent upon nerve impulse flow. It has been suggested by several investigators that newly synthetized DA is preferentially released and that this release is nerve impulse flow dependent. We have therefore studied the effect of AMPH on newly formed ^{3}H-3-MT. AMPH treated rats were injected intraventricularly with ^{3}H-Tyrosine and killed 10 min thereafter by microwave radiations. In contrast to cold 3-MT the striatal concentrations of ^{3}H-3-MT were decreased by AMPH. This finding leads to the following conclusions: 1) cold 3-MT and ^{3}H-3-MT reflect two distinct mechanisms of DA release; 2) it is possible that ^{3}H-3-MT reflects the nerve impulse dependent DA release, being the firing of DA neurons decreased by AMPH. This hypothesis seems to be supported by the observation that when AMPH is given to rats with monolateral lesions of the crus cerebri, a condition that depresses the effect of this drug on firing of DA neurones, no changes of ^{3}H-3-MT levels have been found.

Another experimental condition in which cold 3-MT and ^{3}H-3-MT levels are differently affected, is represented by their post mortem variations. In fact, while the striatal concentrations of cold 3-MT measured 20 min after decapitation of the animals showed a 30 fold increase, ^{3}H-3-MT levels were only slightly affected. Since 20 min after decapitation nerve impulse flow is unlikely present, these data give further evidence that cold 3-MT derives from DA released by mechanisms not dependent upon nerve impulses, while the opposite might be true for ^{3}H-3-MT.

996 DEVELOPMENTAL CHANGES IN THE PHOSPHOLIPID COMPOSITION OF C6 ASTROGLIOMA CELLS IN VITRO Robert V. Dorman and Barry Festoff V. A. Hospital, Kansas City, MO 64128

The maturation of C6 astroglioma cells in culture is correlated with the development of a phospholipid pattern. We followed C6 cells as they developed and reached confluency. The phospholipid composition of these cells changed significantly and the importance of these changes is discussed.

C6 astroglioma cells were grown in MEM with 10% fetal calf serum. The cells were trypsinized prior to passage. Cells were harvested at 3, 6, 24, 48, 72, 96 and 168 hours post-passing. The phospholipids were extracted according to Folch, separated by thin layer chromatography and quantitated by lipid phosphorus. [^{3}H]-thymidine corporation was used to indicate mitotic activity.

The results indicated that ethanolamine plasmalogens (1-alk-1'-enyl-2-acyl-sn-glycero-3-phosphoryl ethanolamines) accounted for approximately 20% of the total phospholipids, considerably more than previously reported. The ratio of plasmalogen to diacyl form of the ethanolamine phosphoglycerides increased from 1.06 at 3 hours to 4.82 at 7 days, indicating considerable changes in ethanolamine phosphoglyceride metabolism as the astroglioma cells pass from growth to stationary phase. During the same time course the mole % of choline phosphoglycerides decreased relative to the other lipid classes. The absolute increase of ethanolamine phosphoglycerides and decrease of choline phosphoglycerides are larger than indicated by the mole % values, because the phospholipid content per cell increased approximately 50% between 3 and 72 hours post-passing. The increasing phospholipid content parellels a decreasing mitotic activity (^{3}H-thymidine incorporation).

These changes in C6 phospholipid composition during growth in culture are summarized as follows: 1) development, as described by the change from exponential growth to stationary phase, correlates with a significant increase in ethanolamine plasmalogens; 2) this alteration is initiated by a dramatic reduction of ethanolamine plasmalogens which coincides with the effects of trypsinization; 3) a relative reduction of choline phosphoglycerides occurs during this same developmental course. The enrichment of choline phosphoglycerides during growth phase and ethanolamine phosphoglycerides during stationary phase may be related to the fluidity or stability of the cell membranes as required for different metabolic states.

997 TRANSPORT OF META-TYRAMINE, PARA-TYRAMINE AND DOPAMINE IN RAT STRIATAL SLICES. L.E. Dyck* (SPON: A.A. Boulton), Psychiatric Research Division, University Hospital, Saskatoon, Saskatchewan, Canada S7N 0W8.

The high affinity uptakes of tritiated meta-tyramine (m-TA), para-tyramine (p-TA) and dopamine (DA) into rat striatal slices (0.2 mm thickness) have previously been shown to be inhibited by 3,4-dinitrophenol (5×10^{-4}M) and ouabain (10^{-4}M). Cocaine (5×10^{-6} M) and low Na^+ (25mM) were also found to inhibit the uptakes of these three amines. Thus, a Na^+-dependent, carrier-mediated transport system, utilizing metabolic energy, perhaps derived from Na^+-K^+ATPase may be involved.

Efflux of a previously accumulated amine was studied by a rapid transfer technique in the presence of nialamide (12.5 μM). The spontaneous efflux curve exhibited a characteristic initial rapid rate of wash-out followed by a secondary slow phase. During this latter phase, if the slices are depolarized with 50mM K^+, a typical release peak was observed. The effect of removal of Ca^{2+} (replaced by 2mM EDTA) from the Krebs Henseleit medium was assessed in the following manner: (1) the removal of Ca^{2+} during both the initial rapid wash-out phase and during the K^+-depolarization; (2) the removal of Ca^{2+} during the K^+-depolarization only; (3) the removal of Ca^{2+} during the second phase in the absence of K^+-depolarization; (4) the addition of cocaine (5×10^{-6}M) during the second phase also in the absence of K^+-depolarization. The first procedure (i.e. expt. 1) was found to cause increased rates of spontaneous efflux of all three amines; but the K^+-elicited effluxes of the tyramines were completely suppressed (i.e. Ca^{2+}-dependent), whereas that of dopamine was only partially suppressed (i.e. partially Ca^{2+}-dependent). This apparent Ca^{2+}-dependency in the case of tyramines was due to the virtual washout of all accumulated amine prior to the depolarization. In expt. 2, the K^+-elicited effluxes of both tyramines were not suppressed, whereas the K^+-elicited efflux of dopamine was again partially suppressed. In expt. 3, the spontaneous effluxes of m-TA and DA were moderately increased, whereas that of p-TA became as large as the K^+-elicited efflux in the first experiment. From the above, it is difficult to assess whether or not the removal of Ca^{2+} suppresses K^+-elicited effluxes. In expt. 4, no change in the spontaneous efflux of DA occurred, and only a small increase was observed for the tyramines. It appears, therefore, that a Ca^{2+}-dependent re-uptake is not responsible for the increased spontaneous efflux caused by Ca^{2+} removal. It may be that Ca^{2+} plays some role in tissue retention of the amines and that this function of Ca^{2+} is of greater importance for p-TA than for m-TA and DA.

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998 OCTOPAMINE: A SUBSTRATE FOR TYPES A AND B MONOAMINE OXIDASE. David J. Edwards and Mark C. Venetti*. Western Psychiatric Inst. & Clinic, Dept. Psychiatry, Univ. Pittsburgh, School of Medicine, Pittsburgh, PA. 15261.

Octopamine was previously reported to be a substrate for only the type A species of monoamine oxidase (MAO) (Houslay & Tipton, Biochem. J. 139:645 [1974]). The recent finding that imipramine, a tricyclic antidepressant drug, causes an increase in octopamine concentrations in rat brain (Harmar & Horn, J.Neurochem. 26:987 [1976]) prompted us to reexamine the substrate specificity of A and B types of MAO towards octopamine, since tricyclics are known to selectively inhibit type B MAO (Edwards & Burns, Life Sci. 15: 2045[1974]). MAO was assayed with [2-^{3}H]-DL-octopamine in .04 M phosphate buffer at pH 7.5. Varying concentrations from 10^{-10} to 10^{-3}M of clorgyline (a selective inhibitor of type A MAO) were preincubated with the enzyme and the reaction was initiated by the addition of substrate. When either rat brain or liver homogenates were used, biphasic inhibition curves were obtained. The clorgyline-sensitive (type A) activities of both the brain and liver enzymes were inhibited 50% at a concentration of $5x10^{-9}$M of the inhibitor. The clorgyline-insensitive (type B) activities of these enzymes were inhibited 50% at an inhibitor concentration of 10^{-5}M. The plateaus of the inhibition curves occurred at 88% and 70% inhibition for the brain and liver enzymes, respectively. In contrast, a single sigmoid curve occurring at high clorgyline concentrations (I_{50}=$7x10^{-5}$M) was obtained for human platelet MAO, a pure type B enzyme. These experiments were repeated with deprenyl, a selective inhibitor of MAO-B. As with clorgyline, the inhibition curves were biphasic for both rat brain and liver MAO and sigmoidal for human platelet MAO. The apparent K_m for octopamine was determined to be $4.8x10^{-4}$M for rat liver MAO. When MAO-A was completely inhibited by preincubation with 10^{-7}M clorgyline, the apparent K_m for the remaining activity was 3.9x 10^{-4}M, indicating that the apparent K_m for octopamine toward MAO-A and MAO-B are similar. In conclusion, these data demonstrate that octopamine is a substrate for both types A and B MAO. Consequently, deamination of this amine may be affected by inhibition of either enzymatic form.

	I_{50} (clorgyline)		I_{50} (deprenyl)	
Enzyme	MAO-A	MAO-B	MAO-A	MAO-B
Human Platelet	-	$7x10^{-5}$M	-	$2x10^{-8}$M
Rat Brain	$5x10^{-9}$M	10^{-5}M	$6x10^{-6}$M	$5x10^{-8}$M
Rat Liver	$5x10^{-9}$M	10^{-5}M	$6x10^{-6}$M	$5x10^{-8}$M

999 INCREASED BRAIN CYCLIC AMP FOLLOWING FOOTSHOCK AGGRESSION IN THE RAT. B. Eichelman, T.M. McMurray*, G. Davis* and A. Qureshi*. Laboratory of Behavioral Neurochemistry, Dept. of Psychiat., Univ. Wisconsin and VA Hospital, Madison, WI 53705.

Rats subjected to electric footshock display two disparate behaviors, escape or aggressive attacks, depending on whether the rat is shocked alone or with a conspecific. Previous work (Eichelman et al., 1976) has shown that whole brain levels of cAMP are increased 100% over control levels for rats subjected to three days of shock-induced fighting (50 footshocks/day of 2 mA intensity, 0.4 s duration, presented every 7.5 s). An increase in whole brain cAMP of only 20% over control levels is present in isolated rats receiving similar footshock.

With paired rats in the footshock paradigm there is a stepwise increase in brain cAMP with each day of shock-induced testing (50 footshocks/day); the increase plateaus at day 3. This elevation, after three days of shock-induced fighting, returns to baseline levels in two weeks. For example, rats footshocked for three days with the parameters described above have brain cAMP levels of .45 pmoles/mg. Unshocked controls have levels of .22. Recovery levels after 1, 3, 5, 10, and 14 days, respectively are: .36, .35, .33, .26, and .27. All animals were sacrificed by focused microwave irradiation. cAMP was assayed by a modification of the protein-binding method of Tovey et al., 1974. Regional changes based on the sevenfold dissection of Glowinski et al. (1966) demonstrated greatest increases of cAMP in regions of the hypothalamus and caudate nucleus. These studies were accomplished with male Holtzman rats of 200 g wt.

Non-fighting Holtzman female rats (180-200 g) do not show the increase in cAMP following paired shock. Rats treated with a monoamine oxidase inhibitor (pargyline, 20 mg/kg/day) show increased levels of shock-induced fighting (Eichelman & Barchas, 1975). Similarly treated rats show an increase in brain cAMP. The time course of this increase over a three day period parallels the development of the facilitated aggression seen with pargyline. (This work was supported by grants from the Medical Research Service of the Veterans Administration Hospital and intramural grants from the University of Wisconsin.)

1000 CYCLIC GMP: A PROPOSED ROLE IN THE PHOTOTRANSDUCTION PROCESS OF PHOTORECEPTOR CELLS. Debora B. Farber and Richard N. Lolley. Dept. Anatomy, UCLA School of Medicine, Los Angeles, CA 90024, and Devel. Neurology Lab., V.A. Hospital, Sepulveda, CA 91343.

Dark-adapted retinal rod outer segments (ROS) of photoreceptor cells possess high levels of cyclic GMP. Upon bleaching of rhodopsin by light, cyclic GMP-phosphodiesterase is activated and the concentration of cyclic GMP falls rapidly. We have investigated purified preparations of bovine ROS in order to evaluate the role of cyclic GMP in modulating the functional biochemistry of ROS. We find protein kinase (PK) activity in the 100,000g supernatant fraction of ROS, which is increased by cyclic nucleotides and which selectively phosphorylates a soluble protein (apparent MW: 30,000 daltons) in a cyclic nucleotide-dependent manner. In the particulate membrane fraction, PK activity phosphorylates opsin independently from cyclic nucleotides. This particulate PK can be solubilized with Lubrol PX and, when in soluble form, it becomes cyclic nucleotide dependent. Conversely, the soluble PK can be reassociated with PK-depleted ROS membranes where it phosphorylates opsin in a cyclic nucleotide-independent manner. Several lines of evidence indicate that the membrane PK and phosphorylation of rhodopsin are associated with sensitivity changes in the ROS (dark/light adaptation). We propose that the soluble PK-30,000 dalton phosphoprotein complex links the light-induced changes in cyclic GMP concentrations with ion permeability changes in the ROS plasmalemma. A model will be presented which illustrates how high levels of cyclic GMP in dark-adapted ROS could allow for the ready access of sodium ions across the plasma membrane (dark-current) and how low levels of cyclic GMP in light-adapted ROS could facilitate a reduction of the dark-current and cause hyperpolarization of the photoreceptor cell. (Supported by NIH Grant EY00395, NSF Grant BMS 74-14784, and the Medical Research Service of the Veterans Administration.)

1001 THE EFFECT OF MAXIMAL ELECTROSHOCK ON ENERGY METABOLITES AND CYCLIC NUCLEOTIDES IN LAYERS OF THE CEREBELLAR VERMIS. G. K. Feussner*, D. W. McCandless*, W. D. Lust and J. V. Passonneau. Laboratory of Neurochemistry, NIH, Bethesda, MD. 20014

Previous studies have shown that maximal electroshock (MES) caused a reproducible seizure response in mice which was reflected in changes in energy metabolites and cyclic nucleotides in the cerebellum. These investigations have been extended to a study of the cerebellar layers to determine whether there was a differential response. The mice were shocked through corneal electrodes with 50 mA for 0.2 sec. The seizure response is characterized by a tonic extensor phase, 0-13 sec; followed by a clonic phase, 13-25 sec, and a subsequent postictal depressive phase. At selected intervals during these periods, the animals were frozen in liquid nitrogen. The dorsal cerebellar vermis was dissected at -30° and sections were cut and frozen-dried according to the method of Lowry and Passonneau (A Flexible System of Enzymatic Analysis, Academic Press, 1972). Samples 0.2-1.2 ug were dissected from the molecular, Purkinje cell-rich, granular and white layers; all samples were from lobultes VI-VII after Larsell. The analyses were made using oil well techniques and enzymatic cycling (Lowry and Passonneau, 1972). Measurements were made of ATP, P-creatine, glucose, glycogen, lactate, GABA, cyclic AMP and cyclic GMP. In general, the response to MES was significantly less in the white matter. P-creatine concentrations showed the most dramatic decrease, falling to 15% of the control values during the excitable phase of the seizure (10 sec). The changes were comparable in all the layers except white matter. The pattern of ATP changes was similar; the maximum decrease at 10 sec was 50% of control. Glucose concentrations decreased only slightly during the excitable phase; however, there was a 2.5-fold increase in glucose 10 min after MES in all layers. The changes in glycogen were delayed, falling to 65% of control value in all layers at 30 sec after MES. Lactate concentrations increased in all layers at 10 sec and continued to increase up to 30 sec after MES. Even after 10 min lactate levels had not returned to control values. There were no significant changes in GABA in any of the layers. Cyclic AMP concentrations in all layers increased 4-fold during the tonic phase and remained elevated at 30 sec after which the levels returned to normal. In contrast, cyclic GMP levels did not change until the postictal depressive period, when the concentrations increased 10-fold. The absolute levels of cyclic GMP and the increases after MES were greatest in the molecular layer. The maximum increases observed 60 sec after MES and were near normal values at 10 min. The effect of phenytoin on metabolite changes after MES was investigated.

1002 DOPAMINERGIC RECEPTORS IN THE CORPUS STRIATUM AND SUBSTANTIA NIGRA-REGIONAL ALTERATIONS IN HUNTINGTON'S DISEASE, J.Z.Fields*, T.D.Reisine*, P.C.Johnson*, L.Z.Stern, and H.I.Yamamura, Univ. of Arizona Health Sciences Center, Tucson AZ 85724.

Neuronal activity in the circuitry connecting the substantia nigra(SN) and corpus striatum(CS) modulates extrapyramidal control of motor function. Progressive loss of neurons as well as biochemical changes have been demonstrated in and may be critical to the tremor and rigidity of Parkinson's Disease and the choreiform movements of Huntington's Disease(HD). In one current model, these symptoms are due to hypo- and hyper-activity, respectively, of nigro-striatal dopaminergic(DA) neurons which synapse on and inhibit cholinergic interneurons in the CS.

Using ligand binding assays, DA receptors have been demonstrated in the CS but not in the SN. Recently(Fields et al., 1977, Trans.Amer.Soc.Neurochem., 8, 193) we developed an assay to label brain DA receptors using (+)-butaclamol-displaceable ^{3}H-spiroperidol binding. We have measured DA receptors in rat brain(CS=540 fmol/mg prot; SN=1.24 fmol/mg tissue) and in human brain(CS=245 fmol/mg prot; SN=16 fmol/mg prot). Although evidence has been accumulating suggesting that, within the SN, dopamine is released from the dendrites of nigro-striatal DA neurons onto axons of incoming striato-nigral neurons, the functions of these DA sites in the SN are uncertain.

In HD, the density(but not ligand affinity or sensitivity to (+)butaclamol) of DA receptors in the caudate nucleus and putamen are decreased by over 50%($P<.05$). Parallel decreases in choline acetyltransferase activity in these areas suggests: 1) a loss of cholinergic interneurons in the CS, and 2) that at least some of the DA receptors are postsynaptic on the cholinergic interneuron cell bodies.Substantial decreases in DA receptors were also found in the globus pallidus(-53%), frontal cortex(-72%), occipital cortex(-72%) and amygdala(-66%). Data on DA receptors in the SN of HD are too preliminary to draw any conclusions.

To further examine the DA receptors in the CS and SN we have used kainic acid lesions which are thought to selectively destroy neuronal cell bodies. Kainate injection in the CS induces biochemical changes in that area that resemble those seen in HD and suggest that kainate lesion of the CS may serve as a useful animal model for HD. Lesions of the CS and SN may also yield information bearing on the location and function of the DA receptors in the SN. Supported in part by grants from the NIMH(MH-27257) and the Committee to Combat Huntington's Disease and from an RSDA (MH-00095) ot HIY and a USPHS Postdoctoral Fellowship (MH-05248-01) to JZF.

1003 PARTIAL REVERSAL BY THAM OF THE EFFECTS OF HYPOXIA ON NEUROTRANSMITTER METABOLISM. G.E. Gibson, M. Shimada and J.P. Blass. MRRC, UCLA Medical School, Los Angeles, California 90024.

Previous studies in our laboratory and elsewhere have demonstrated that the synthesis of acetylcholine (ACh) is closely linked to the oxidation of carbohydrates; impairing carbohydrate oxidation in vivo or in vitro leads to proportional impairment of ACh synthesis, even though the flux to ACh is less than 1% of that through oxidation. We recently found that the earliest changes accompanying mild anemic hypoxia (induced with $NaNO_2$) are a decrease in the incorporation of [U-^{14}C]glucose into ACh and an increase in cyclic GMP with no change in cyclic AMP. These changes preceded the change in whole brain lactate.

The purpose of the present investigations was to determine if prior treatment with THAM (trihydroxyaminomethane, 10 mmol/kg) at an alkaline pH (10.3) would ameliorate certain gross behavioral and biochemical changes accompanying hypoxia induced with $NaNO_2$ (1.1 mmol/kg). Alkaline THAM significantly delayed the time until loss of righting reflex (from 21±1 to 27±2 min) and until death (from 24±1 to 32±2 min). Values are mean ± SEM; n=25 for controls and 12 for THAM treated; $P < 0.01$ for both measurements. Animals treated with an equivalent amount of THAM at an acid pH (4.5) were indistinguishable from controls.

Alkaline THAM also ameliorated some biochemical effects of hypoxia induced with $NaNO_2$ (1.1 mmol/kg). In these experiments, mice were injected with 3 nCi/kg of [U-^{14}C]glucose and 20 µmol/kg of [$^{2}H_4$]choline 19 min after the injection of $NaNO_2$ and 1 min before sacrifice by microwave irradiation. THAM ameliorated several effects of hypoxia (values are means ± SEM per mg brain protein; numbers of animals are in parentheses):

	[$^{2}H_4$]-ACh pmol	[U-^{14}C]-ACh DPM	Total ACh pmol	cGMP pmol	Lactate nmol	Total Choline pmol
Control (9)	5.1±0.7	31±3	166± 9	0.5±0.1	14±1	230±17
$NaNO_2$ alone (9)	0.7±0.1	4±1	117±16	1.3±0.3	59±3	675±85
$NaNO_2$-THAM (9)	2.1±0.3	8±2	161±17	0.7±0.2	41±6	355±22

Hypoxia increased the amount of [$^{2}H_4$]choline in the brain (19±3 to 50±7 pmol/mg prot) and THAM partially reversed the change (to 33±4). cAMP did not change under the conditions of these experiments.

These studies demonstrate that THAM at an alkaline but not an acid pH can partially reverse the effects of hypoxia. The mechanism of action of THAM is not yet known but seems to involve its potential action as a buffer. (This research was supported in part by NICHD grant HD-06576, Public Health Service Grant RR-05756-03 from the NIMH, and grant 17691 from MH.)

1004 ISOLATION OF NERVE GROWTH FACTOR FROM HUMAN PLACENTAL TISSUE. L. D. Goldstein*, C. P. Reynolds* and J. R. Perez-Polo (SPON: J. R. Perez-Polo). Dept. Human Biological Chem. & Gen., Univ. of Tex. Med. Br., Galveston, TX 77550, U.S.A.

Nerve growth factor (NGF) is a multimeric protein having an essential role in the development and maintenance of vertebrate sensory and sympathetic neurons as well as being necessary for axonal sprouting in adrenergic structures of the central nervous system under certain experimental conditions. Mouse submaxillary gland and snake venom NGF are the only two molecular species of NGF which have been well characterized. The protein complex isolated from these sources contains up to three non-identical subunits (α, β, and γ) which are of similar molecular weight but display different isoelectric points. The NGF activity resides strictly with the basic β-NGF subunit. Although levels of NGF activity have been detected in human tissues by the chicken dorsal root ganglion assay and by the radioimmunoassay using antibodies directed against mouse β-NGF, no successful isolation of human NGF has ever been reported. Human placental tissue has been investigated as a suitable source from which NGF may be isolated. Biological activity employing the chicken embryo dorsal root ganglion assay was determined for the amnion, cord serum, fetal, maternal and mixed bloods, and placental cotyledonary tissues. Only the amnion and placental cotyledons showed significant levels of NGF activity. Applying techniques standardized for mouse NGF purification, a fraction rich in NGF activity (20 BU/mg) was isolated at neutral pH from placental cotyledons. Analysis by thin layer isoelectric focusing in polyacrylamide gel (TLIEF) revealed a basic protein species which when eluted from the gel demonstrated NGF activity. NGF activity was not detected in any other part of the pH gradient. Fractionation of the complex isolated at neutral pH by preparative sucrose gradient isoelectric focusing also yielded a basic protein which demonstrated NGF activity (1-3 nanograms/BU). A basic protein species having NGF activity was also isolated from a preparation of human amnion tissue by preparative isoelectric focusing on a granulated gel slab. Supported by NINDS grant NS14034, Welch Grant H698, a gift from the Brown & Lupton Foundation to J.R.P. and a McLaughlin Fellowship to L.D.G.

1005 SPECTRAL STUDIES OF CROTOXIN AND ITS SUB-UNITS. Michael R. Hanley.* (SPON. M.Calvin). Lab.Chem.Biodynamics & Dept. Mol.Biol., Univ. of Calif., Berkeley, Ca. 94720 USA.

Crotoxin, a protein neurotoxin from Crotalus durissus terrificus (Brazilian rattlesnake) venom, is a complex of an acidic polypeptide (crotoxin A) and a basic polypeptide (crotoxin B). Lethality of the complex results from synergistic interaction of the two components. Crotoxin A is not toxic. Crotoxin B is weakly toxic and has phospholipase A2 activity. To study how crotoxin A influences the B component, we have investigated the characteristics of their complex by fluorescence and circular dichroism (CD) spectroscopy. The crotoxin complex is dissociated at acid pH. Over the pH range 2 to 4, there was a 70% attenuation in intrinsic fluorescent intensity and a concomitant shift of the emission maximum from 345 nm to 338 nm. Several near-UV CD bands, tentatively assigned to tryptophan residues on crotoxin B, increased in magnitude and shifted to longer wavelengths. These data are consistent with "burying" of crotoxin B tryptophans in the complex. Evidence of larger structural changes with complex formation was obtained from the far-UV spectra. From pH 2 to 4, the proportion of ordered secondary structure increased (28% to 40% α-helix and 18% to 45% β-structure). Computer summation of the individual contributions of the isolated sub-units' CD agreed well at pH 2, but gave a substantial underestimate at pH 4. Complex formation also stabilized against heat and guanidine hydrochloride denaturation. These results suggest that the complex is not only stable, but also conformationally unique at neutral pH. Exposure of toxin to 80% trifluoroethanol (conditions thought to mimic membrane environments) produced dissociation of the complex. Similar treatment of crotoxin A produced a conformational change reducing the proportion of ordered structure. A comparable structural transition in the A sub-unit may be induced by contact with the physiological target membrane, thereby releasing the active B sub-unit. In this way, crotoxin A would act as a delivery vehicle for crotoxin B and increase its relative lethality by more selective targeting. In view of the requirement of both the enzymatic and synaptic-blocking activities for calcium, we have investigated the effects of divalent cations on spectral parameters. A CD signal at 303 nm (assigned to crotoxin B tryptophan) gave a dose-dependent increase to twice control values with increasing calcium concentration at neutral pH. The calcium effect was strongly pH-dependent and was abolished at pH 4. The data are consistent with a calcium-binding site similar to those reported in other phospholipase A2 enzymes. (Supported by Division of Biomedical and Environmental Research of US Energy and Development Administration).

1006 ION-EXCHANGE/FLUOROMETRIC MEASUREMENT OF AMINO ACIDS IN HUMAN CEREBROSPINAL FLUID. T.A. Hare and B.S. Glaeser, Thomas Jefferson University, Philadelphia, PA

Measurement of amino acids and related amines in human cerebrospinal fluid (CSF) has been limited because of the relatively low concentration of most of these components in CSF. Using conventional amino acid analysis with ninhydrin detection, approximately 30 components can be readily measured (Perry et al., J. Neurochem. 24, 587, 1975; Lakke and Teelken, Neurology 26, 489, 1976) although when 10-fold concentrates of CSF have been utilized the number of detectable components is increased to about 40 (Dickinson and Hamilton, J. Neurochem. 13, 1179, 1966). Recently with the introduction of the fluorogenic reagent orthophthalaldehyde (OP) (Roth and Hampai, J. Chromatogr. 83, 353, 1973), it has become possible to increase the sensitivity of amino acid analysis by several orders of magnitude. Human CSF specimens which had been obtained from patients with various neurological disorders and stored at -70°C, were pooled, lyophylized to dryness, and then taken up to 0.07 times the original volume with water. This preparation was deproteinized using 0.4 N perchloric acid. Aliquots of 0.25 ml of the deproteinized solutions (equivalent to 1.7 ml of CSF) were analyzed for their amino acid content. The analytical procedure utilized a 6 mm ID column which contained a 60 cm bed of spherical cation exchange resin. The column was eluted with a 0.15 N lithium citrate buffer pH 2.9 for 475 min followed by a 0.15 N lithium citrate buffer pH 4.00 for 225 min followed by a 1.10 N lithium citrate buffer pH 4.60 for 510 min. Primary amines were detected in the eluate using the fluorogenic OP reagent. When these analyses were carried out at a relatively low sensitivity setting on the fluorometer 45 components were readily distinguishable including peaks corresponding to those previously described in the literature. When the analyses were carried out at a 10-fold higher sensitivity setting, an additional 35 peaks were apparent which had been evident only as trace constituents in the previous run. Chemical and biological characterization of these components may provide valuable new information about the functioning of the central nervous system.

1007 THE EFFECTS OF MAGNESIUM DEFICIENCY ON THE LEVELS OF DOPAMINE, NOREPINEPHRINE, AND SEROTONIN IN THE RAT BRAIN AND HEART. Mary Daniel Healy*, Jose A. Perez*, Audelio Rivera*, David Limon* and Dolores Chacon* (SPON: A. T. Modak). Incarnate Word College of The United Colleges of San Antonio, San Antonio TX 78209.

The neuromuscular involvement in the magnesium deficiency (MGD) syndrome in rats has been well documented in the literature. The animals exhibit signs of neuromuscular hyperirritability, often culminating in generalized seizures and death. The biogenic amines--dopamine, norepinephrine, and serotonin--play an important role in the nervous system as neurotransmitters. Although the exact functions of these biogenic amines have not been elucidated, their levels have been shown to correlate with changes in a rat's susceptibility to seizures. In this study, the effect of a MGD diet on the levels of dopamine, norepinephrine, and serotonin in the rat brain and heart was investigated. Groups of male albino rats weighing 90-110g at the beginning of the experiment were placed on MGD and control diets. The biogenic amines of the brain and heart in experimental and control animals were analyzed spectrophotofluorometrically at 5 day intervals for 20 days.

The concentration of norepinephrine and serotonin were found to be progressively depleted in the brain and heart tissue in dietary magnesium deprivation. The dopamine levels in the brain, however, were higher than the control values on days 5 and 15, and lower on days 10 and 20. This dopamine graph correlated directly with the levels of hyperexcitability as measured in magnesium deficiency.

It is thought that the hyperexcitability of the nervous system in MGD may be due to altered neuronal membrane stability. We have attempted to clarify some of the possible pathogenically important chemical changes underlying the neurologic manifestations of the MGD syndrome in young rats and to obtain data on the quantitative interrelationship between concentration of electrolytes in the brain and heart and the concentrations of the neurotransmitters--dopamine, norepinephrine and serotonin--in these two organs. Support by NIH Grant RR-08077.

1008 EFFECT OF STRESS ON LSD-INDUCED DISAGGREGATION OF BRAIN POLYSOMES. J. Heikkila*, L. Holbrook* and I. Brown* (SPON: C. K. Govind). Dept. of Zoology, Scarborough College, Univ. of Toronto, West Hill, Ontario, Canada M1C 1A4.

Previously we have reported that brain polysomes are disaggregated after intravenous injection of LSD to young rabbits (Holbrook and Brown, J. Neurochem., 27,77,1976). The effect is brain specific and due to a decreased rate of reinitiation of protein synthesis. We now report that LSD produces a stress-like response in that plasma corticosteroids are elevated and that application of physiological stress accentuates the extent of LSD-induced brain polysome disaggregation. One hour after administration of LSD (50-100 ug/kg) plasma corticosteroid levels were increased by 2.5-3.0 fold over saline controls. Three forms of physiological stress were applied in addition to LSD (25 ug/kg): physical restraint (immediately after drug injection), hypoglycemia (previously starved for 24 hr.) or epinephrine bitartrate (25 ug/kg concomitant with LSD). All three forms of stress greatly accentuated the level of LSD-induced disaggregation of brain polysomes and also elevated plasma corticosteroids. Stress treatment without LSD did not effect brain polysomes. Sedation with the general anaesthetics, ethanol (4 gm/kg) or pentobarbital (20 mg/kg) before LSD (100 ug/kg) resulted in a near total inhibition of polysome disaggregation. Animals placed in an unfamiliar environment after LSD injection showed a greater disaggregation of brain polysomes than rabbits returned to their home cages. These results suggest that elements of physiological stress, arousal and environment influence the degree to which brain protein synthesis is affected.

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1009 ROLE OF THE CHOROID PLEXUS IN ENHANCING THE CEREBROSPINAL FLUID SINK EFFECT DURING UREMIA. Mark A. Hise* and Conrad E. Johanson* (SPON: J. W. Woodbury). Dept. Pharmacology Univ. of Utah Col. Med., Salt Lake City, Ut. 84132

The uremic state is associated with a progressive change in the concentration of electrolytes, urea and metabolic toxins in the blood. Such an alteration in blood composition is a potential threat to the finely-regulated composition of the extracellular fluid (ECF) of the C.N.S. An enhanced cerebrospinal fluid (CSF) sink action (due to an increase in CSF secretion) on substances leaking into the brain could serve to protect the brain ECF during uremia.

To test the hypothesis that there is an enhanced CSF sink effect during uremia, the following experiments were done to ascertain the activity of the Na-K pump (Na-K-ATPase) system in the choroid plexus (CP). Uremia was induced in adult male rats by bilateral ligation of the renal pedicles for periods of 1,8,16, 24,32 or 48 hr. At the termination of the experiment, lateral ventricular CP, blood and cisternal CSF were sampled and analysed for Na and K. Values given below are in mEq/kg wet, expressed as the ratio of Na/K.

Uremic state for:	Plasma	CP	CSF
1 hr	143/4.5	52.1/101	152/2.86
8 hr	143/6.4	54.4/100	154/3.13
16 hr	141/7.0	55.8/106	155/3.21
24 hr	139/7.5	59.7/106	155/-

The trend of a progressive increase in both Na and K in CP as well as in CSF continues at 32 hr and 48 hr post nephrectomy; similarly, the trend in plasma Na and K noted for the initial 24 hr continues for the subsequent 24-hr-period.

The changes in CP electrolytes are apparently a reflection of an enhanced activity of the Na-K-ATPase in this secretory tissue. Since an augmentation of Na-K-ATPase activity in the CP is generally associated with a concomitant increase in CSF secretion, it is tempting to conclude that there is an increased CSF sink action during uremia due to a greater bulk flow of CSF through the ventricular system. The mechanism of Na-K pump stimulation is presumably due to a build-up in CSF K during uremia. (Supported by NIH Grant-GM00153.)

1010 DOPAMINERGIC FUNCTION AFTER ETHANOL WITHDRAWAL Paula L. Hoffman * and Boris Tabakoff. Department of Physiology, School of Medicine, University of Illinois, Chicago, Illinois 60612

Previous work in our laboratories demonstrated a decreased sensitivity of central dopaminergic (DA) receptors in mice withdrawn from chronic ethanol treatment. Animals were less responsive to the hypothermia induced by several doses of the DA receptor agonist, piribedil, for up to seven days following ethanol withdrawal, and this change was paralleled by a reduced response of dopamine-sensitive adenylate cyclase to stimulation by dopamine in the ethanol-withdrawn animals. The time course of disappearance of these functional alterations in the DA systems followed that of disappearance of tolerance to ethanol. To further elucidate the possible relationship between modifications in dopaminergic function and development of physical dependence on ethanol, the time course of the appearance of changes in DA- sensitive adenylate cyclase activity was studied. At the time of withdrawal, adenylate cyclase was stimulated by DA to an equal degree in both ethanol- treated and control animals; however, by eight hours after withdrawal, when ethanol was eliminated from the animals, sensitivity to dopamine stimulation had decreased significantly. Differences in adenylate cyclase activity were more prominent in brain areas containing the nigro-striatal system compared to the mesolimbic systems. Enzymes from ethanol-treated and control animals were, however, equally stimulated by Na fluoride, indicating no change in total amount of adenylate cyclase activity. To determine whether the decreased sensitivity to dopamine stimulation reflected a decreased density of dopaminergic receptors or changes in coupling between receptor and adenylate cyclase, specific binding of ^{3}H-dopamine to brain regional preparations from both ethanol-treated and control animals was characterized by Scatchard analysis. The results of these studies will be discussed.

This work was supported in part by the NIAAA and the St. of Ill. Dept. of Mental Health. B.T. is a Schweppe Foundation Fellow.

1011 EFFECTS OF OSMOLARITY ON RESPIRATION AND FINE STRUCTURE *IN MITO*CHONDRIA FROM MATURE AND IMMATURE RAT BRAIN. D. Holtzman*, M. M. Herman*, M. Desautel* (SPON: K. A. Kelts). Stanford Univ. Sch. Med., Stanford, CA, 94305

We recently have shown that isolated mature rat brain mitochondria respond to changes in ambient osmolarity very differently than liver mitochondria (submitted for publication). Polarographic study of brain mitochondria in hypo-osmolar media showed inhibited State 3 (ADP-dependent) respiration with a NAD-linked substrate pair, glutamate and malate, and with succinate. In hyperosmolar media there was an increase in State 4 (ADP-independent) respiration and only a transient inhibition of State 3 with the NAD-linked substrates. With succinate as substrate, respiration was not affected by moderate increases in osmolarity. In contrast, liver mitochondria showed increased State 4 respiration in hypo-osmolar media and inhibited State 3 in hyperosmolar media with both substrates. Electron microscopy showed that liver mitochondria were markedly swollen in hypo-osmolar media and very condensed in hyperosmolar media, while brain mitochondria swelled less in hypo-osmolar media and, in hyperosmolar media, half the mitochondria were condensed and half showed no morphologic changes compared to controls.

In hypo-osmolar media, five day old rat brain mitochondria showed respiratory changes similar to those seen in the adult. However, in contrast to the adult, five day old rat brain mitochondria showed persistent inhibition of State 3 respiration with both NAD-linked substrates and succinate in hyperosmolar media. The spontaneous reversal of State 3 inhibition with NAD-linked substrates and the loss of inhibition with succinate in hyperosmolar media appeared progressively with maturation and reached adult levels by 30 days. During this same maturational period, brain mitochondria resistant to changes in fine structure in hyperosmolar media progressively appeared. At five days almost all brain mitochondria were condensed in the hyperosmolar media. By 30 days approximately half the mitochondria showed little or no morphologic change in hyperosmolar media. The age-dependent appearance of this "osmolar resistant" sub-population of brain mitochondria is coincident with astroglial proliferation and may be important in maturation of the brain's resistance to edema-producing insults (e.g., heavy metal encephalopathy and seizures).

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1012 PHENYTOIN-INDUCED STIMULATION OF THE Na-K PUMP IN THE CHOROID PLEXUS - CEREBROSPINAL FLUID SYSTEM. Conrad E. Johanson* and Quentin R. Smith* (SPON: S.A.Turkanis) Dept. Pharmacology, Univ. of Utah Col. Med., Salt Lake City, Ut 84132.

Determination of the effects of the anticonvulsant drug, phenytoin (PT), on the distribution of Na and K among various compartments in the C.N.S. is important because of relevance not only to convulsive disorders but also to other electrolyte-related disorders (cerebral edema, hydrocephalus, etc.). The ratio of Na:K in the CSF is 50:1; since PT stimulates Na-K-ATPase activity maximally when Na:K is 50:1, it has been hypothesized that PT enhances the turnover of Na and K across the CSF-facing membrane of the choroid plexus (CP).

On the basis that a change in the rate of transport of ions across a cell membrane is reflected by a change in the cell concentration of those ions, we have tested the above mentioned hypothesis as follows: Adult rats were injected i.p. with PT at a dose of either 10 or 40 mg/kg (or appropriate vehicle) and sacrificed 1 hr later for analysis of tissue electrolytes. In response to the 40 mg/kg treatment, the [K] in CP (lateral ventricle) increased by 11% to 101 mEq/kg while [Na] decreased by 6% to 47 mEq/kg. At the 10 mg/kg dose, there was also a statistically significant rise in CP [K] and a fall in [Na] but neither change was as great as that in the 40 mg/kg animals. CSF [K] varied more-or-less inversely with dose of PT; however, PT did not significantly alter CSF [Na]. In both cerebral cortex and cerebellum there was a slight rise in [K] and a similarly small reduction in tissue [Na]; however, such changes in electrolytes in these regions of the brain were not significantly different. Since PT did not alter plasma [K], and because the [K] in erythrocytes decreased following PT administration, the rise in CP [K] after PT treatment cannot be accounted for by a change in the electrolytes in residual blood of the sampled tissue; a similar conclusion can be drawn about the decrease in CP [Na]. The extracellular fluid volume in the CP, as measured by the distribution of tritiated-inulin, increased progressively with dosage of PT.

Thus, the electrolyte and the radioinulin space data for the CP, together with the observed changes in CSF [K], constitute evidence in support of the hypothesis that PT stimulates a Na-K pump in the CP.

(Supported by a grant from the Epilepsy Foundation of America and by a grant from the Faculty Research Committee, Univ. of Ut.)

1013 HISTOCHEMICAL DISTRIBUTION OF HEXOKINASE IN THE NORMAL AND ISCHEMIC GERBIL BRAIN. S. Kakari*, K. Nishimoto*, J. Walker* and M. Spatz. (SPON: M. Nirenberg) NIH, Bethesda, MD 20014.

Hexokinase (HK) catalyzes phosphorylation of glucose to glucose-6-phosphate - a control step in glycolysis. A histochemical evaluation of the brain HK in gerbils, both normal and those subjected to unilateral common carotid artery occlusion and release, has been undertaken in order to obtain additional information about the ischemic sequelae of carbohydrate metabolism.

In the normal gerbil, HK-reaction is distributed in increasing order: cerebral cortex, hippocampus, basal ganglia in both hemispheres symmetrically. Activity appears strong in the gray but weak in the white matter. In gray matter, HK is variable strong in both neuronal perikarya and the neuropil. The most intense HK-reaction is seen in ependyma and choroid plexus, while capillaries show a moderate activity.

In unilateral cerebral ischemia HK diminishes first in hippocampus followed by the basal ganglia and cortex, proportional to the duration of the post-ischemic period (1-20 hrs) following one hour occlusion. The loss of HK in basal ganglia, first seen at 3 hours progressively extends to encompass almost the whole region by 20 hours post-release. By contrast, enhanced HK may also be seen in various structures including (a) pyramidal cells in the H3 sector of hippocampus at 5 hours and (b) axonal swellings and reactive glia in the white matter adjacent to hippocampus 20 hours post-release.

The regional and cellular changes in hexokinase activity demonstrated by these studies may add another dimension in the evaluation of changes in the regional cerebral glucose consumption seen in the ischemic gerbil brain (Kakari et al., J. Neuropath. Exp. Neurol. 36:680, 1977).

1014 DOPAMINE SYNTHESIS IN SYNAPTOSOMES: EFFECT OF CALCIUM REMOVAL WITH EGTA. Gregory Kapatos* and Michael J. Zigmond. (SPON: B. Dixit). Dept. of Life Sciences, University of Pittsburgh, Pittsburgh, PA 15260.

Exogenous Ca^{++} has been reported to inhibit, stimulate, or have no effect on soluble preparations of striatal tyrosine hydroxylase (TH). Ca^{++} has also been shown to inhibit dopamine (DA) synthesis by striatal tissue slices. These inconsistant findings prompted us to examine the effect of Ca^{++} on DA synthesis by striatal synaptosomes, a system which offers a natural physical state and pterin cofactor for TH but does not retain the intact neural circuitry of the tissue slice. Synaptosome-rich P2 fractions were incubated in the presence of L-(1-^{14}C) tyrosine (TYR) in a Kreb's-Ringer phosphate buffer at a final pH of 6.2 (optimal) or 7.2 which contained either 0.87 mM Ca^{++} (control medium), no Ca^{++}, or no Ca^{++} with 0.5 mM EGTA. DA synthesis was determined by collecting $^{14}CO_2$. The removal of Ca^{++} without chelation produced a small decline in DA synthesis (11%), when determined in the presence of 8 µM medium TYR. The removal of Ca^{++} and the addition of EGTA produced an elevation in synthesis under these conditions which was greater at pH 7.2 than at 6.2 (40 vs. 18%). By varying medium TYR concentration we determined that this stimulation of synthesis was due entirely to an increase in Vmax without an alteration in Km TYR regardless of medium pH. (Under control conditions Vmax decline as pH was increased but again no change in Km TYR was observed). Although the stimulation of synaptosomal DA synthesis by Ca^{++} displays characteristics which appear identical to the effect of db-cAMP, we have determined that these treatments do not act by a similar process. In contrast to stimulation by db-cAMP, stimulation by Ca^{++} removal did not alter the ability of exogenous DA (1 µM) to inhibit synthesis. Moreover, Ca^{++} removal antagonized the stimulation of synthesis by db-cAMP (1 mM) and also partially reversed the ability of db-cAMP to overcome synthesis inhibition by DA. Soluble TH prepared from synaptosomes which were stimulated by Ca^{++} removal was not in an activated state, although less TH was found associated with synaptosomal membranes. These data suggest that Ca^{++} plays some role in the activation of TH by db-cAMP but that the removal of Ca^{++} does not in itself alter the characteristics of TH in striatal synaptosomes. Supported in part by a grant from the USPHS (MH-20620).

1015 DISTRIBUTION OF ANGIOTENSINOGEN IN RAT BRAIN. John A. Lewicki*, James H. Fallon, and Morton P. Printz*. (SPON: Arnold Miller). Depts. of Med. and Neurosci., Univ. of Calif., San Diego, La Jolla, Calif. 92093.

Recent evidence has accumulated for the existence of an independent central renin-angiotensin system. However, the mechanisms and sites of angiotensin generation within the CNS are not yet understood. One approach toward answering this question is to examine the properties of angiotensinogen, the specific prohormone of angiotensin I, within the brain. We have recently demonstrated that angiotensinogen is present in whole brain homogenates in significant concentrations and that it is physicochemically similar to plasma angiotensinogen (Fed Proc. 35:770, 1976). The purpose of the present study was to investigate the regional distribution of this prohormone throughout the brain. Sprague-Dawley rats were perfused with saline to remove all blood from the central compartment and the brains extracted and sectioned in a Minotome. A punch technique was used to isolate 32 specific brain regions. The tissues were then homogenized and incubated with excess kidney renin (37°, pH 6.0) to generate angiotensin I which was measured by radioimmunoassay. The highest angiotensinogen levels were localized to the area postrema, periventricular hypothalamus, and organum vasculosum lamina terminalis. Further studies, however, indicated that these areas appeared erroneously high due to the relative deficiency of a proteolytic enzyme activity (angiotensinase) which degraded the angiotensin I generated at the incubation pH. This angiotensinase activity was not inhibited by EDTA, phenylmercuric acetate, phenylmethylsulfonylfluoride, or any combination thereof. However, the angiotensinase was separated from angiotensinogen by micro-precipitation of the latter with 2.3M ammonium sulfate. Reassay of the extracted angiotensinogen disclosed a more uniform distribution of the prohormone throughout the brain. Only the pituitary, parietal cortex, and cerebellum were relatively deficient in the prohormone, while the periventricular hypothalamus and several other midline structures were slightly elevated over the remainder of the areas. Further characterization of the prohormone in the various regions is currently underway. We conclude that angiotensinogen is not restricted to only a few sites in the CNS, but rather is widely distributed. Based on these findings, the function of angiotensinogen within the brain remains unclear. (Supported by NIH HL 15808).

1016 EFFECT OF SMALL INCREASES IN EXTRACELLULAR [K^+] ON PROTEIN SYNTHESIS IN THE HIPPOCAMPUS: LOCALIZATION AND MECHANISM. Peter Lipton* and Catherine J. Heimbach*, (SPON: D.D. Gilboe). Dept. of Physiology, Sch. Med., Madison, WI 53706

Small increases in extracellular [K^+] ([K^+])$_{e.c.}$) increase the rate of protein synthesis in the guinea pig hippocampus maintained in vitro (Lipton & Heimbach, J. Neurochem., in press). We have postulated that this effect may be one way by which cerebral electrical activity increases protein synthesis in situ and thus may be related to biochemical plasticity. Here we report further characterizations of the effect.

A. LOCALIZATION OF THE EFFECT. 1. Tissue Specificity. Increasing [K^+]$_{e.c.}$ from 3.0 to 6.4mM increases [^{14}C]-lysine incorporation into protein of cerebral cortex to the same extent as into the hippocampus (29%); however it has no effect upon incorporation into kidney cortex, liver or heart slices. 2. Cell Specificity. We measured the effect of increasing [K^+]$_{e.c.}$ on [^{14}C]-lysine incorporation into sub-cortical white matter. Basal rates of incorporation into this tissue were similar to those of hippocampus but [K^+]$_{e.c.}$ had no effect upon incorporation suggesting that [K^+]$_{e.c.}$ affects protein synthesis in nerve cells and not in glia or capillary endothelial cells. 3. Intracellular Localization. Effects of [K^+]$_{e.c.}$ on incorporation into crude subcellular fractions was assessed. Incorporation into the soluble (105,000g supernatant) protein was unaffected by [K+]$_{e.c.}$. The greatest effect upon incorporation was in the crude mitochondrial and nuclear fractions. (% increases in synthesis: Homogenate 20±5; Nuclear 27±12; Mitochondrial 29±6; Cytosol 3±5, n=8 experiments.

B. MECHANISM OF THE EFFECT. 1. Transcription or post-transcription. Increasing [K^+]$_{e.c.}$ from 3.0 to 6.4mM increased [^{3}H]-uridine incorporation into RNA by 14%, suggesting that its effect upon protein synthesis might be mediated by an effect on transcription of RNA. However, when tested in the presence of a combination of α-amanitin and actinomycin D at concentrations which decreased [^{3}H]-uridine incorporation into RNA by 97% [K^+]$_{e.c.}$ exerted its normal effect upon protein synthesis. This strongly suggests that the effect is exerted post-transcriptionally. 2. We measured the effect of increasing [K^+]$_{e.c.}$ upon intracellular [K^+]. Increasing [K^+]$_{e.c.}$ from 3.0 to 6.4 increased intracellular [K^+] by 7±3%. This small increase suggests that increased cell [K^+] is not the basis for the increase in protein synthesis. [K^+]$_{e.c.}$ thus appears to exert a tissue specific effect, in vitro, upon cerebral protein synthesis - apparently increasing synthesis of (some of) the non-soluble neuronal proteins. The effect appears to be exerted post-transcriptionally and not to be mediated by increased intracellular [K^+]. Supported by NSF.

1017 PARTIAL CHARACTERIZATION OF RADIOLABELED α-BUNGAROTOXIN SPECIES AND THEIR INTERACTION WITH MEMBRANE-BOUND ACETYLCHOLINE RECEPTORS. Ronald J. Lukasiewicz*, Michael R. Hanley* and Edward L. Bennett. Lab. Chem. Biodynamics, Univ. of Calif., Berkeley, CA, USA 94720.

Toward preparation of a suitable toxin-label for the nicotinic acetylcholine receptor in brain, studies concerning structural and functional properties of radiolabeled α-bungarotoxin derivatives were undertaken. ^{125}I is incorporated into a single tyrosine residue of α-bungarotoxin (α-Bgt) at ca. 85% yield, using a non-enzymatic iodination procedure. Mono-iodinated ([I_1]α-Bgt) and di-iodinated([I_2]α-Bgt) derivatives are purified and separated from native toxin on Whatman CM-52, 5mM $NaPO_4$, pH 6.5 with gradient elution (0.02-0.1M NaCl). Catalytic reduction of [I_1]α-Bgt in the presence of tritium gas yields tritiated α-Bgt ([^{3}H]α-Bgt) at ca. 90% recovery which is chromatographically and spectrally indistinguishable from native toxin. Mono-iodinated α-Bgt may be differentiated from native and [^{3}H] α-Bgt species on the basis of ultraviolet absorption (UV) and circular dichroism (CD) spectra. UV and CD spectra of [I_2]α-Bgt are markedly altered relative to native α-Bgt, exhibiting strong positively dichroic UV absorbtion at 320nm and little or no dichroism at 230nm. These differences in chromatographic and UV and CD spectral properties are suggestive of a small conformational change accompanying iodine incorporation, and a larger change in three dimensional structure of α-Bgt on introduction of a second iodine atom into an exposed tyrosine residue.

That these structural changes are manifest as differences in toxin binding characteristics is shown in preliminary experiments using membrane-bound nicotinic acetylcholine receptor (nAChR) derived from Torpedo electroplax, and rat brain crude mitochondrial fractions (CMF). As determined from log probit analyses, native α-Bgt is 3-6 times more effective than cold [I_1] -Bgt and [I_2]α-Bgt towards competing for [$^{125}I_1$]α-Bgt and [$^{125}I_2$]α-Bgt binding sites in rat brain CMF. Rank order of α-Bgt derivative inhibition effectiveness is preserved for binding to electroplax nAChR, but differences in absolute effectiveness are reduced relative to that for CMF α-Bgt binding sites. Values of K_d determined from saturation binding assays are ca. 10nM for electroplax nAChR, independent of the form of radiolabeled α-Bgt, while K_d for specific binding to rat brain CMF is ca. 0.8nM for[^{3}H]$_d$α-Bgt, 1.0nM for[$^{125}I_1$] α-Bgt, and 3.0nM for [$^{125}I_2$] -Bgt. These results suggest that there might be differences in the toxin binding active site for nAChR derived from peripheral and central sources, in addition to the established differences in radiolabeled α-Bgt derivatives. Supported by Div. of Biomed. and Environ. Research of ERDA. RJL is a postdoctoral fellow of NINCDS.

1018 QUANTITATION OF CELLULAR PROTEINS BY COMPUTER ANALYZED TWO DIMENSIONAL POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE). W.A. Lutin*, W.W. Carley*, and J.A. Freeman. Dept. Biochem. and Anat., Sch. Med., Vanderbilt Univ., Nashville, TN. 37232.

Previous work has shown (Freeman, Nature, 1977, in press) that chronic binding of cholinergic receptor protein in the optic tectum of the toad B. marinus with α-bungarotoxin is associated with plastic modifications of retinotectal synapses. In order to study the dynamics of receptor protein and other cell surface proteins during synaptogenesis, we have developed a method for quantitating cell proteins separated by 2-dimensional PAGE, using a modification of O'Farrell's method (JBC 250: 4007, 1975). Cellular proteins from tectum and retina homogenates were extracted with phenol, dialyzed, and run on 2-dimensional PAGE. Tectal gels contained over 400 clearly separated protein components, ranging in molecular wt. from 2000 to 500,000 daltons, and in pI from 4 to 8 pH units. Gel proteins were detected either by autoradiography following incorporation of ^{14}C-amino acids, or with Coomassie blue. Autoradiograms or photographs were scanned with a digital densitometer. The resulting 1000x1000 point image was analyzed with a Sigma-7 computer. Image details were displayed as a 2-D reconstruction, a 3-D perspective display revealing relative concentration of protein peaks, or as iso-density contour maps. The numbers, positions, and concentrations of individual proteins were determined by a spatial convolution technique. Individual protein spots were characterized by a 2-dimensional Gaussian least squares best fit. Using orthopthalaldehyde labeled protein standards, we have been able to estimate concentration, isoelectric pH and molecular weight for essentially all of the proteins resolved by the 2-D gel separation. To compare the protein spectra of two or more gels, their Gaussian parameterized representations were first normalized with respect to electrophoretic mobility and concentration, and then differenced. The resulting residual surfaces clearly revealed differences in protein expression. This method is being used to quantitate changes in different proteins during degeneration and regeneration of retinotectal synapses.

1019 ^{3}H - CHOLINE UPTAKE IN ABDOMINAL GANGLIA OF LIMULUS POLYPHEMUS. M.A. Maleque* and James G. Townsel, Department of Physiology, Meharry Medical College, Nashville, Tennessee 37208.

Nervous tissue is unable to synthesize choline (Ch) (Ansell and Spanner, 1971, Biochem. J. 110: 201, Schwartz et al., 1975, J. Gen. Physiol. 65: 255) an immediate precursor of acetylcholine (ACh). Thus, the supply of Ch for ACh synthesis is functionally important. A high affinity (HA) sodium dependent transport system presumably localized in cholinergic nerve terminals has been described. Moreover, this Na^+ dependent HA transport system is closely associated with the synthesis of releasable ACh.

ACh has been implicated as a neurotransmitter in Limulus polyphemus (Garry, 1942, Am. J. Physiol. 136: 182; Stephens and Greenberg, 1973, Histochem. Cytochem. 21: 923; Townsel et al., 1976, Neurosci. Abs. 2: 618). Choline acetyltransferase activity has been demonstrated in the brain as well as the abdominal ganglia of Limulus (Emson et al., 1974, J. Neurochem. 22: 1089; Malthe-Sorenson and Emson, 1976, J. Neurochem. 27: 341). The current study was undertaken to determine the kinetics and nature of choline uptake in Limulus abdominal ganglia.

The results indicate dual uptake systems for Ch within the abdominal ganglia of Limulus. One, a HA system with K_m 4 x 10^{-6} M and V_{max} 7.5 pmole/10 min./mg of tissue in the concentration range of 1 x 10^{-7} - 1 x 10^{-6}M and another a low affinity (LA) system with K_m 3.3 x 10^{-4} and V_{max} 38.64 pmole/10 min./mg of tissue in the concentration range of 1 x 10^{-6} - 1 x 10^{-4}M. In the HA system Ch uptake was decreased by 78% in the absence of Na^+. Maximal Ch uptake was achieved at a concentration of 0.44 M Na^+ which is the Na^+ concentration in Limulus physiological saline (Chao, 1933, Biol. Bull 64: 358). The replacement of K^+ and Ca^{2+} resulted in nominal inhibition of Ch transport (i.e. 17% and 18% inhibition respectively in the HA system and 3% and 19% inhibition within the LA system). The addition of 2.5 mM glucose did not enhance uptake. The pharmacological sensitivities of these uptake systems will be presented. The presence of a Na^+ dependent HA system within the abdominal ganglia of Limulus suggests the presence of cholinergic neurons within this structure.

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1020 HETEROGENEITY OF BRAIN TUBULIN SUBUNITS AND CHARACTERIZATION OF LOW MOLECULAR WEIGHT ASSOCIATED PROTEINS. Charles A. Marotta, Jamie L. Harris,* Aesta A. Manschreck,* and Jeffrey M. Gilbert. Harvard Med. Sch. and Mailman Res. Center, McLean Hospital, Belmont, Ma. 02138

Microtubular protein was isolated from rat forebrain by biochemical purification (ammonium sulfate precipitation followed by DEAE cellulose chromatography) or by two cycles of aggregation - disaggregation. The protein subunit structure was examined on two-dimensional electrophoretograms: first dimension, urea isoelectric focusing gel; second dimension, sodium dodecyl sulphate exponential acrylamide slab gel. Two forms of α tubulin were separated in the second dimension on the basis of different rates of migration (α_1 and α_2). Each of these species were further differentiated into at least two subunits with distinct isoelectric points. β Tubulin was separated into a major species (β_1) and a minor species (β_2). The same results were obtained using protein from either purification method. Molecular weights were determined: the α_1 and α_2 groups of proteins have approximate sizes of 58,000 d. and 57,000 d. respectively. β_1 migrated slightly ahead of β_2 (53,300 d. and 54,600 d., respectively) We have consistently observed the presence of low molecular weight proteins that co-purify with tubulin. These consist of a single species of 70,000 d. and four proteins in the size range of 34,000-36,000 d. Artefactual results were ruled out by detection of the multiple forms of α and β tubulin as well as the associated proteins in a two-dimensional electrophoretogram of total supernatant proteins from rat brain; on this gel we observed that the single major soluble protein of rat brain is the β_1 subunit of tubulin. In summary, the subunit structure of brain α and β tubulin is more complex than previously reported; low molecular weight tubulin associated proteins co-purify with the major α and β proteins. Separation and visualization of multiple forms of α and β tubulin on two-dimensional electrophoretograms is consistent with reports that provide evidence for post-translational modification of these proteins. The gel system described should make feasible the isolation of individual species for chemical analysis. (This work was supported in parts by USPHS Grant MHI6674, a Mellon Faculty Award to C.A.M., and NIMH Research Scientist Development Award 3K01 MH-70713 to J.M.G.)

1021 PURIFICATION AND CHARACTERIZATION OF THY-1 ALLOANTIGEN FROM MOUSE BRAIN. Larry D. McClain and Ronald T. Acton. Diabetes Research and Training CTR., UAB, Birmingham, Alabama 35294.

The fractionation of mouse brain plasma membranes by zonal centrifugation techniques has yielded a preparation high in the expression of Thy-1 alloantigen. Enzyme marker assays reveal this membrane fraction to be rich in Na^+, K^+ dependent, ATPase activity although distinct from fractions expressing 2', 3'-cyclic nucleotide 3'-phosphohydrolase, a putative marker for myelin and glial plasma membranes. This suggests that Thy-1 in brain is localized on membranes of neuronal origin. Immunoferritin labeling of isolated synaptosome preparations substantiates the suggestion that this cell surface antigen may represent a specific marker for synaptic plasma membranes. Thy-1 has been purified from C57Bl/6J mouse brain and physico-chemically characterized. Acetone precipitation of membrane protein is followed by extraction with deoxycholate. Purification is accomplished utilizing a Lens culinaris lentil lectil affinity chromatography column followed by gel filtration on Ultrogel AcA 44. SDS gel electrophoresis of the eluted peak of Thy-1 activity reveals a single band of 25,000 molecular weight. Amino acid and carbohydrate compositions of Thy 1.2 purified in this way are compared with the values for rat Thy 1.1 and mouse lymphoblastoid cell Thy 1.1 and Thy 1.2.

1022 ACTIVATION OF TYROSINE HYDROXYLASE IN INTACT, ISOLATED ADRENAL CHROMAFFIN CELLS. *John A. Meligeni*, John W. Haycock and Jack C. Waymire*. Department of Psychobiology, University of California at Irvine, CA 92717 and Department of Psychology, University of California at Riverside, CA 92502.*

Tyrosine hydroxylation was investigated in 3-7 day old cultures of intact chromaffin cells isolated from bovine adrenal medulla. Activity was monitored by measuring $^{14}CO_2$ evolution from cells incubated (15', 37°C) in a calcium-deficient HEPES buffer system (pH7.2) containing 10^{-5}M 1-^{14}C-tyrosine. Under these conditions the basal level of hydroxylation was 5-10 pmole tyrosine/15 min/10^6 cells.

Addition of 2.5 mM $CaCl_2$ to the medium resulted in a 100-200% increase in hydroxylation. Although without effect in a calcium-deficient medium, both 10^{-5}M acetylcholine (ACh) and 50 mM KCl increased the effect of calcium. In addition, 8-bromo-3',5'-adenosine monophosphate potentiated the effects of either ACh plus $CaCl_2$ or KCl plus $CaCl_2$ but not the effect of $CaCl_2$ alone.

Similar studies were conducted on a partially purified fraction of tyrosine hydroxylase from bovine adrenal medulla. In the presence of pteridine cofactor and DOPA decarboxylase (to release $^{14}CO_2$),none of the above agents increased hydroxylation.

These studies suggest that in the physiological situation both calcium and cyclic AMP may play roles in regulating tyrosine hydroxylase activity. However, the mechanisms by which these agents modify tyrosine hydroxylase appears to be disparate and of an indirect nature. The relationship of such alterations in tyrosine hydroxylase activity to stimulus-secretion coupling processes is currently being investigated.

1023 A NOVEL POTENTIOMETRIC ESTIMATION OF MONOAMINE OXIDASE ACTIVITY USING AN AMMONIA SELECTIVE ELECTRODE. L.R.Meyerson, K.D. McMurtrey*, B.L. Pashkoff* and V.E. Davis. V.A. Hospital and Baylor College of Medicine, Houston, Tx 77211.

A sensitive and convenient method for the estimation of monoamine oxidase (MAO) activity in rat brain homogenate and mitochondrial preparations based on measurement of ammonia produced by oxidative deamination of amine substrates by an ammonia selective electrode has been developed. The electrode (Orion Res. Model 95-10) employs a hydrophobic membrane which is permeable to gas and separates the solution measured from the electrode filling solution. The ammonia in the sample diffuses through the membrane until NH_3-N partial pressures are at equilibrium and the resulting potential (mV) is recorded with a mV/pH meter (Orion Res. Model 701-A). Assay mixtures consist of 0.05 M sodium pyrophosphate (pH 8.1), tissue preparations containing 0.3 - 5.0 mg protein, 1.0 mM freshly prepared substrate and triple distilled deionized water in a total volume of 2.0 ml. After incubation at 37.5°C for 30 min., the reactions are terminated by the addition of 4 ml of pH 12.0 Titrisol buffer (E.M. Labs). The electrode is then immersed in the stirred reaction mixtures and the mV potentials are recorded. The amount of ammonia generated enzymatically is obtained by calculating the difference in ammonia concentration in control (substrate added after incubation) and test mixtures. Ammonium chloride is used as the reference standard for preparation of calibration curves. The sensitivity of this method is comparable to radiometric, fluorometric and polarographic techniques. Thirty nmoles of ammonia formed from a substrate in the assay mixture could be measured accurately. This response is obtained with 0.3 mg mitochondrial or 0.5 mg homogenate protein. The Δ mV potential corresponding to ammonia formed from substrate by MAO in brain homogenate and mitochondrial preparations is linear to enzyme protein concentration and to time during the 30 min. incubation period. The stoichiometric relationship between the amounts of ammonia production and amine depletion, measured by high pressure liquid chromatography (Anal. Biochem. 72: 566, 1976), is evidenced by the equal amounts of ammonia formed and substrate deaminated. The assay technique offers diverse utility. Numerous primary amine substrates can be employed with this method. The procedure is also useful for enzyme kinetic analyses, comparison of substrate specificity and the study of enzyme inhibitor interactions. Compared to many other assay procedures, the method outlined offers the advantages of sensitivity, simplicity, accuracy and rapidity. (Supported by USPHS Grant AA 00226 and the Veterans Administration).

1024 THE EFFECT OF CEREBRAL ISCHEMIA AND POSTISCHEMIA ON MONOAMINE OXIDASE ACTIVITY. Dejan Micic*, Igor Klatzo and Maria Spatz (SPON: B. B. Mrsulja). Lab. Neuropath. & Neuroanat. Sci, NIH, Bethesda, MD 20014.

In our previous experiments, we had described the cerebral ischemic and postischemic reduction of biogenic amines and the accumulaton of its metabolites being due to (1) increased synthesis and (2) possible outtransport inhibition but not due to altered monoamino oxidase (MAO) activity in the brain (Acta neuropath. 36, 1, 1976 and Brain Res. 98, 388, 1975). In order to elucidate further the metabolic fate of these substances we investigated the cerebral MAO activity itself during ischemia and postischemia of Mongolian gerbils.

Several groups of anesthetized animals (pentobarbital 20 mg/kg i.p.) were subjected to unilateral carotid artery occlusion for 1 hour and various periods of release or to continuous occlusion fr 1-5 hours. The mitochondrial MAO activity was assayed by microfluorimetric method (Bioch. Pharmacol. 14, 1686, 1965).

The activity of MAO was significantly reduced in the hemisphere ipsilateral to 1 hour occlusion and release for 20 and 72 hours and 1 week as compared to the contralateral, to the one from control and sham operated animals (ischemic = 16.05 + 1.35 (7), 12.99 $\pm$ 1.59 (7), 13.23 $\pm$ .76 (6) nmoles 4HOQ/mg P/hr, respectively; control = 21.91 $\pm$ .79 (12) nmoles 4HOQ/mg P/hr). Normal levels of the enzyme were found 4 weeks after the release of 1 hour occlusion. In the continuously ischemic gerbils the MAO activity was found to be only reduced at 5 hours (15.79 + 1.0 (7) nmoles 4HOQ/mg P/hr).

These results substantiate our previous postulate that the MAO activity couldn't be responsible for the decreased levels of biogenic amines and/or increased accumulation of its metabolites in ischemic and postischemic brains.

1025 ELECTRICAL ACTIVITY AND ENERGY METABOLISM IN THE ISOLATED TOAD BRAIN - ROLES OF GLYCOLYSIS AND OXIDATIVE METABOLISM. D. F. Moffett* and J. C. LaManna. Department of Physiol. Pharmacol., Duke Univ. Med. Cntr. Durham, N.C. 27710.

Isolated toad brains provided with pyruvate respond to electrical stimulation with an initial decrease in fluorescence intensity corresponding to a net oxidation of reduced nicotinamide adenine dinucleotide ($NADH_2$). The period of net oxidation lasts about 30 sec. and is followed by return to or overshoot of the resting fluorescence baseline. The reductive overshoot is exaggerated and prolonged if glucose is substituted for pyruvate. If pyruvate is the exogenous substrate, addition of the glycolysis inhibitor iodoacetate abolishes the reductive overshoot while sparing the initial oxidative response. If glucose is the substrate provided, iodoacetate abolishes fluorescence transients immediately and initiates a transition to an increased steady-state fluorescence. Replacement of pyruvate by succinate also abolishes the oxidative transient. After change to succinate, each train of pulses is followed by a transition to an increased fluorescence level until after several trains a new steady-state level is attained. Return to pyruvate results in a decrease in steady-state fluorescence and recovery of the oxidative transient. These results are interpreted as follows: The initial oxidative transient reflects the response of terminal oxidative metabolism, while the later increase in fluorescence is due to activation of glycolysis. The increased fluorescence in the presence of glucose + iodoacetate is not readily explained but might be due to diversion of glucose metabolism into the pentose phosphate pathway, with consequent reduction of nicotinamide adenine dinucleotide phosphate (NADPH). The abolition of oxidative transients of $NADH_2$ by succinate is attributable to monopolization of electron flow through phosphorylation site I of the respiratory chain by succinate with possible reverse electron flow adding to the increase in $NADH_2$.

In the absence of electrical stimulation, pyruvate-glucose, pyruvate-lactate and pyruvate-succinate changes fail to evoke changes in the steady-state level of fluorescence, suggesting that during rest access of exogenous substrate to the reactions of intermediary energy metabolism is restricted.

1026 HYPERTHERMIA IN INFANT RATS: EFFECTS ON BRAIN POLYSOMES AND AMINO ACID LEVELS IN BRAIN AND BLOOD. L.L. Murdock* and F.L. Siegel. Depts of Pediatrics and Physiological Chemistry, Center for Health Sciences, University of Wisconsin, Madison, WI 53706.

Brain polysomes prepared from infant rats (7-days old) exposed to elevated ambient temperature (39.5°C for 45 min) are highly disaggregated. Disaggregation is evident 15 min after the animals have entered the hot environment and is nearly maximal after 25 min. Substantial recovery of polysomes is seen 20 min after hyperthermic rats are transferred from 39.5°C to 33°C ambient temperature. After 45 min exposure to 39.5°C ambient temperature the levels of certain amino acids, particularly essential amino acids, rise markedly in brain. Amino acids whose levels rise under these conditions are: phenylalanine (388%), valine (387%), leucine (340%), arginine (320%), isoleucine (262%), lysine (181%), histidine (136%), methionine (110%), threonine (84%), tyrosine (61%), serine (47%), glycine (43%), glutamic acid (17%). The levels of glutamine, aspartic acid, taurine, γ-aminobutyric acid and citrulline did not change under these conditions. These increases in brain amino acid levels were reversed within an hour after the rats were transferred from the hot environment to 33°C ambient temperature. The levels of amino acids in blood plasma also rise during hyperthermia; these rises are smaller than those in brain and are less selective but are likewise reversed by returning the animals to 33°C ambient temperature. Although the mechanism of the rises in blood and brain amino acid levels and their relationship to polysome disaggregation remain to be elucidated, it seems likely that the rises may have significant consequences for a) the functional status of protein synthesis in brain; b) the levels of neurotransmitter substances which are derived from amino acid precursors in brain; and c) intermediary metabolism in brain. (Supported by PHS grant HD 09045).

1027 THE EFFECT OF ACUTE SPINAL CORD INJURY ON AMINO ACIDS IN THE THORACIC CORD OF THE CAT. JL Osterholm, TA Hare, BS Glaeser, JD Irvin, JL Alderman. Depts. of Neurosurgery and Pharmacology, Thomas Jefferson Med. Coll., Phila. Pa. 19107.

The levels of amino acids (AA) including the putative inhibitory AA neurotransmitters glycine (Gly) and GABA and excitatory transmitters aspartate (Asp) and glutamate (Glu) were determined in the spinal cord of the cat one hr following 500g/cm injury to the thoracic region (T-8). A 2 cm cord sample containing the injury site and immediately adjacent regions was subdivided into 2mm sections and AA were measured on an AA analyzer after conversion to a fluorescent derivative of O-phthaldehyde.

Neither Gly nor GABA were altered in either the site or adjacent regions. There was a decrease in both Asp and Glu at the site although this was not statistically significant. Alanine, Isoluecine and leucine were elevated 330, 320 and 280% (all $p < .05$) respectively at the site and returned to control values 3mm remote to the injury. Phenylalanine, Tyrosine and several basic AA were also elevated above normal levels within the site proper. These data suggest that neurons containing the inhibitory AA transmitters are relatively unaffected by severe cord trauma one hr post-injury. In contrast, profound and highly site restricted aberrations in behavior occur in those AA generally considered as part of the metabolic pool.

1028 INCREASED PERMEABILITY OF PERIVENTRICULAR TISSUES IN LEAD POISONING. L.A.O'Tuama, C.S.Kim*, J.D.Mann* and J.T.Gatzy*. Univ. N. Carol. Sch. Med., Chapel Hill, N.C. 27514

IV administered pulses of ^{210}Pb accumulate selectively in neural barrier tissues (O'Tuama et al. Toxicol Appl Pharmacol, 35, 1-9, 1976) and the accumulation of tyrosine by choroid plexus is inhibited by the addition of lead nitrate in vitro (Kim et al. Environ Health Perspectives, in press, 1977). These results suggest that the barrier tissues may be a "target organ" for neural effects of lead. The present study was designed to define further the interaction of inorganic lead with the neural barrier tissues. Adult mongrel cats were assigned to control or poisoned groups. The poisoned group was pretreated with lead carbonate 600mg qd for 5 days. On the sixth day the animals were anesthetized and subjected to ventriculocisternal perfusion with mock CSF containing inulin with or without added ^{210}Pb (approx.600cpm/ml). Blood lead concentrations (means ± SD) averaged 91.5 ± 72μg/100ml in the pretreated group and only 3.65 ± 2.76μg/100ml in controls. However tissue lead levels between control and poisoned differed only for the paraventricular tissues (.18 ± .02 vs. .42 ± .18μg/gram) ($P<.05$). CSF formation did not differ in controls and treated animals (.021 ± .006 vs. .026 ± .002ml/min). The net clearance (k_o) of ^{210}Pb from the perfused ventricle was strikingly greater in the pretreated group (ml/min: controls .022 ± .005; poisoned 3.24 ± 1.55) ($P<.01$). Whereas % of ^{210}Pb recovered in the cisternal collection of control animals exceeded by 4-fold that in the poisoned group ($P<.05$). The increased clearance of ^{210}Pb from CSF in the pretreated group may have influenced the deposition of the metal within neural tissue since whole brain recovery of ^{210}Pb in pretreated cats was only 1/2 that found in control animals. The question arises whether the increased removal of the tracer from the ventricle in the poisoned group is due to an increase in overall permeability of the paraventricular tissues or to occupation of tissue binding sites by endogenous lead with resultant changes in complexing of the metal by the tissues. Preliminary results suggest that an increased ventricular clearance of ^{14}C labeled urea accompanies the high extraction of ^{210}Pb in the pretreated group, suggesting that these animals show a generalized increase in permeability of the periventricular tissues. These studies support our hypothesis that the neural barrier tissues may be a target site for inorganic lead. Furthermore, this interaction occurs in a species showing minimal behavioral reaction to lead and before detectable elevation of neural tissue levels. Lead-induced barrier dysfunction may offer a model for the mechanisms of neurotoxicity associated with "low level" lead exposure (David et al. Lancet 3:900-903, 1972.) Supported by grant from NIEHS #5 R01 ES01151.

1029 CHARACTERIZATION OF A PEPTIDE DERIVED FROM THE SERUM OF PSYCHIATRIC PATIENTS. R. M. Palmour*, F. R. Ervin, H. Wagemaker*, and R. Cade*. Dept. Genetics, U California, Berkeley, CA 94720; NPI, UCLA, Los Angeles, CA 90024; Dept. Psychiatry, U Louisville, Louisville KY 40202; Dept. Medicine, U Miami, Miami, FL 33124.

Wagemaker and Cade (Amer. J. Psych., 1977, in press) have reported that hemodialysis leads to clinical improvement in a group of drug-resistant psychiatric patients. Dialysate #1 (from a series of sixteen weekly dialyses) contains greater than 100-fold concentrations of a particular peptide as compared to dialysate #16. We have isolated the oligopeptide (MW approximately 3000) from the dialysate of several different patients, and have quantitated its disappearance in dialysate #1, #8, and #16. Furthermore, the quantity of peptide present in serum before and after dialysis has been determined for several patients. The quantity of peptide in a patient who showed clinical deterioration following cessation of dialysis was determined, as was the quantity of peptide present in dialysate #16 of a patient whose clinical improvement was ambiguous. Amino acid composition and amino acid sequences have been determined for the specific peptide derived from the serum and/or dialysate of each patient. The potential utility of this peptide as a marker for a sub-category of psychiatric disease, and as a predictor for a specific therapeutic regimen, will be discussed.

1030 PHOSPHORYLATION OF PROTEINS IN THE SQUID GIANT AXON
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Proteins in the squid giant axon were labeled with ^{32}p by (1) incubation of intact axons in artificial sea water containing [γ-^{32}P]-ATP or $^{32}PO_4$; (2) intracellular infusion of the axons with the radioactive ATP or PO_4; and (3) by in vitro incubation of isolated axoplasm with radioactive ATP or PO_4. When the intact axon was incubated with extracellular [γ-^{32}P]-ATP, twice as much ^{32}P-labeled protein appeared in the axoplasm than in the sheath as was determined using the extrusion technique. The two major phosphorylated peaks in axoplasm had molecular weights of 400 x 10^3 and 200 x 10^3, whereas in the sheath the major phosphorylated protein was 12 x 10^3 daltons. The phosphorylation of the 200 x 10^3 dalton protein in axoplasm was decreased when cAMP was added to the axoplasm and [γ-^{32}P]-ATP in vitro; or in situ, when the axon was subjected to repetitive electrical stimulation in the presence of [γ-^{32}P]-ATP. The 12 x 10^3 dalton protein increased in phosphorylation, both in axoplasm and sheath, during repetitive electrical stimulation. The significance of these results will be discussed.

1031 FATTY ACID COMPOSITION OF SUBCELLULAR FRACTIONS DERIVED FROM A VITAMIN B_{12}-DEFICIENT BRAIN.
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Subcellular fractionation and subsequent lipid isolation has been carried out on brain from a patient who suffered a cellular deficiency of the 5-deoxyadenosylcobamin and methylcobalamin co-enzyme forms of vitamin B_{12}. Adenosylcobalamin is required for the enzyme methylmalonyl-CoA mutase. Reduced activity of this enzyme results in an accumulation of methylmalonyl-CoA and propionyl-CoA, potential precursors for branched-chain and odd-chain fatty acids, respectively. Examination of the fatty acid composition of choline and ethanolamine glycophospholipids indicated a relative enrichment of odd-chain fatty acids which were identified by gas-liquid chromatography-mass spectroscopy as C_{15}, $C_{15:1}$, C_{17}, and $C_{17:1}$. A mixture of methyl branched C_{17} fatty acids was also identified. These odd-chain fatty acids were found in all the subcellular fractions examined. Soluble, microsomal and mitochondrial fractions were isolated from both gray (GM) and white (WM) matter, and myelin which was only isolated from white matter. The affected brain phospholipids generally had a lower unsaturated fatty acid content than the control brain phospholipids. Odd-chain fatty acids accounted for 8.5-13.2% of the total fatty acid in choline phospholipid compared to control values up to 1.2%.

Phosphatidyl Choline Odd-Chain Fatty Acid Content (% Total Fatty Acids)

	Soluble	Microsomes	Mitochondria	Myelin
Control				
WM	1.2	Trace	Trace	Trace
GM	1.1	0.8	0.5	---
Patient				
WM	10.2	12.0	11.5	9.8
GM	13.2	9.7	8.5	---

Examination of the myelin sphingolipids, sphingomyelin, cerebroside and sulfatide, yielded abnormal fatty acid profiles. The sphingomyelin contained only small amounts of $C_{24:1}$ fatty acid. Both normal and hydroxy fatty acid-containing cerebroside and sulfatide had reduced levels of C_{24} fatty acid. Determination of the relative hydroxy and normal fatty acid content of these galactolipids indicated an abnormally high hydroxy fatty acid level. If the abnormal sphingolipid fatty acid profiles are characteristic of vitamin B_{12} deficiency is unknown. Further cases will need to be analyzed to establish this fact.

1032 OXIDO-REDUCTION OF NICOTINAMIDE ADENINE DINUCLEOTIDE IN NEURONS FOLLOWING EXCITATION DURING CARBON DIOXIDE PARTIAL PRESSURE CHANGES.
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It has been reported (Rodríguez-Estrada, Am J Physiol 228:996, 1975) that reduced nicotinamide adenine dinucleotide (NADH) level of dorsal root ganglion neurons change after a short period of peripheral nerve stimulation in aerobic conditions, the changes observed were an initial decrease (oxidation) followed by an increase of NADH level (reduction). In this work it was expected that NADH rate of oxido-reduction of dorsal root ganglion neurons should changed after rising or lowering the intracellular pH due to change of oxido-reduction potential of NADH/NAD couple of pyridine-linked dehydrogenases. Fluorometric NADH determinations were done on in vitro preparations of dorsal root ganglion neurons of frogs (Rana palmipes spix). In vitro preparation was placed in a moist chamber at a constant temperature of 25°C. This chamber was provided with an outlet and inlets for gases. The peripheral nerve was stimulated with square pulses (0.2 ms duration, 20 p/s, 5 sec stim). The pH changes were induced by circulating moistened micture of carbon dioxide in oxygen. NADH level of the preparation increased after oxygen was replaced with a CO_2/O_2 mixture (CO_2= 2.5%, O_2= 97.5%), afterwards replacing the gas mixture with oxygen, the NADH level returned to that level observed before. The transient changes observed on the preparation following peripheral nerve stimulation were blocked after lowering the pH by replacing the oxygen with CO_2/O_2 mixture. Afterwards, changing the CO_2/O_2 mixture with oxygen, the transient changes on the NADH level of the preparation were observed again. The transient changes were blocked, first, NADH reduction and later on NADH oxidation, and they returned again in the same order. The increase of NADH after a decrease of pH was attributed to a decrease of the rate of oxido-reduction of the mitochondrial respiratory chain, and this decrease of oxido-reduction rate was also observed in the transient changes induced during neuron excitation. Decrease in the rate of respiration will decrease the energy supply of the neuron.

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1033 A RAPID ASSAY FOR FEMTOMOLE QUANTITIES OF DOPAMINE, NOREPINEPHRINE, AND EPINEPHRINE IN BIOLOGICAL SAMPLES.
Charles F. Saller* and Michael J. Zigmond. Dept. of Life Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have developed a method for the analysis of the catecholamines (CAs), dopamine, norepinephrine, and epinephrine, based upon a procedure originally described by Engelman et al. (Amer. J. Med. Sci. 255:259, 1968), and later modified by Coyle and Henry (J. Neurochem. 21:61, 1973) and Cuello et al. (J. Neurochem. 21:1337, 1973). The CAs are converted to their O-methylated derivatives, 3-methoxytyramine (3-MT), normetanephrine (NMN), and metanephrine (MN), by the enzyme catechol-O-methyltransferase (COMT; EC 2.1.1.6) with ^{3}H-S-adenosylmethionine serving as the methyl donor. Samples are deproteinized in 0.1N $HClO_4$ and the supernatants containing up to 1 ng of total CA are incubated for 10 min at 37°C with 10 mM $MgCl_2$, 6 mM EGTA, 11 mM dithiothreitol, 0.3 mg COMT [purified according to Nikodejevic et al. (J. Pharmacol. Exp. Ther. 174:83, 1970) through the ammonium sulfate precipitation steps and freeze dried], 0.5 µc S-adenosylmethionine (100 nM) and 0.5 M Tris acetate buffer, pH 9.1, in a final volume of 30 µl. The incubation is stopped by placing tubes on ice, adding 2 µl of 2.5 M $CaCl_2$ to inhibit COMT and 10 µl 20% phosphotungstinic acid in 2N HCl (2:3) to precipitate unreacted S-adenosylmethionine. (Blanks receive $CaCl_2$ prior to incubation.) Precipitates are sedimented by brief centrifugation and the supernatants are spotted on the absorbent layer of silica chromatography plates (LQ6DF, Kontes) followed by 5 µl of a carrier solution containing 5 µg 3-MT, 2.5 µg NMN, and 2.5 µg MN in 0.2N acetic acid. The plates are developed in the dark for 90 min using chloroform:methanol:ethylamine (16:3:2) (De Prada and Zürcher, Life Sciences 19:1161, 1976). The spots, corresponding to 3-MT (Rf = 0.83), NMN (0.45), and MN (0.64), are visualized under UV light and scraped into scintillation vials. The silica is eluted overnight with 10 ml Econofluor scintillation cocktail (New England Nuclear) containing 2.5% di-(2-ethylhexyl) phosphoric acid, to which is added 400 µl 0.5 M borate buffer, pH 10.5. This assay offers a number of advantages over the original procedures: First, it permits the simultaneous determination of all three CAs, with little or no cross contamination or interference by CA precursors, metabolites, or a variety of drugs including amphetamine, 6-hydroxydopamine, and α-methyltyrosine. Second, it is highly sensitive, yielding 20-30,000 CPM/ng for each CA with a blank of <500 CPM, and thus can quantify 300 fmols (50 pg) or less of these compounds. Third, it is extremely simple and rapid and can be used to analyze 100 tubes or more in a few hours. Supported in part by a grant from the USPHS (MH20620).

1034 DEVELOPMENTAL CHANGES IN REGIONAL DISTRIBUTION OF CENTRAL NERVOUS SYSTEM CARBONIC ANHYDRASES. Victor S. Sapirstein*, Michael C. Trachtenberg and Marjorie B. Lees. E.K. Shriver Ctr., Waltham, MA 02154 and Boston VA Hosp., Boston, MA 02130.

Brain carbonic anhydrase (CA) is a predominantly glial enzyme whose major function concerns fluid and ion movements. The concentration of CA is known to vary with species, age and brain region. The present study was undertaken to further define the regional distribution of CA and to differentiate the developmental patterns of the soluble, membrane-bound and myelin forms of the enzyme.

Long-Evans rats were perfused rapidly with heparinized saline and the brain and spinal cord dissected at 4°. Total, soluble, membrane and myelin fractions were prepared by standard procedures. Assay for lactic dehydrogenase showed no contamination of the myelin by the soluble fraction.

In adult animals (90 days), the total CA activity per gm fresh tissue showed an antero-caudal progression with upper brain stem 2250 units, lower brain stem 1600, cerebellum 1080 and spinal cord 375. Activity in the upper brain stem was therefore more than 5 times higher than in spinal cord. A similar pattern was observed in 75, 37 and 16 day old animals but the upper brain stem values decreased with age; upper brain stem/ spinal cord ratios were 5, 3 and 1, respectively. The ratio of lower brain stem/ spinal cord similarly decreased from 4 at 90 days to slightly more than 1 at 16 days. This pattern reflects the delayed development of anterior regions as compared with spinal cord. The soluble and membrane-bound forms of the enzyme each showed a developmental pattern similar to that of the total enzyme. Furthermore, the relative contribution of soluble and membrane-bound CA remained constant at the ages studied. Analyses of discrete anatomical regions reinforce the relative invariance in the percentage of membrane-bound CA. White matter regions exhibited significantly higher activity than did gray matter regions, while maintaining a similar neuraxial progression. These observations suggest that CA plays an important role in white matter fluid dynamics.

The specific activity (units/mg protein) of the myelin obtained from different regions of the CNS also showed an rostro-caudal progression: upper brain stem 12, lower brain stem 6, cerebellum 5 and spinal cord 2. However, in contrast to the other fractions, the specific activity of the myelin remained constant between 16 and 90 days. The myelin CA thus appears to be under separate developmental control and probably represents a distinct form of the enzyme.

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1035 DISTRIBUTION OF TYROSINE HYDROXYLASE, CHOLINE ACETYLTRANSFERASE, AND GLUTAMIC ACID DECARBOXYLASE IN THE RAT STRIATUM. Michael C. Scally,* Ismail H. Ulus,* and Richard J. Wurtman. Laboratory of Neuroendocrine Regulation, MIT, Cambridge, MA 02139.

Recent reports describe the distribution of dopaminergic innervation and receptors within various parts of the striatum. In the present study, we mapped the rostral-to-caudal distribution of the neurotransmitter-synthesizing enzymes tyrosine hydroxylase (TOH), choline acetyltransferase (CAT), and glutamic acid decarboxylase (GAD) in striatum. Coronal sections of caudate-putamen of 400 µm width were taken beginning with the most rostral part (9700 µm, according to Konig and Klippel) of the striatum and extending to the most caudal part (6100 µm). The distribution of these enzymes varies markedly within the striatum. TOH and CAT were present primarily in the rostral part of the striatum (2.50 vs. 1.06 nmoles $^{14}CO_2$ formed per mg protein per hour for TOH, and 52.7 vs. 33.8 nmoles ^{14}acetylcholine formed per mg protein per hour for CAT at 9300 and 6500 µm, respectively), while most of the GAD activity was present caudally (117.4 vs. 198.6 nmoles $^{14}CO_2$ formed per mg protein per hour at 9300 and 6500 µm, respectively). The relative activities of TOH and CAT paralleled each other throughout the length of the striatum. These data provide further evidence for reciprocal innervations by cholinergic and dopaminergic neurons in the striatum (Ulus, I.H. and Wurtman, R.J., Science 194:1060, 1976). (These studies were supported in part by a grant from ADAMHA, MH-28783.)

1036 EFFECT OF FLUPHENAZINE ON HIGH AFFINITY CHOLINE UPTAKE IN VITRO AND ON ACETYLCHOLINE AND CHOLINE LEVELS IN STRIATUM AND CORTEX. Kathleen A. Sherman*, Israel Hanin, and Michael J. Zigmond (SPON: R. J. Ertel). Depts. Psychol., Psychiat. (WPIC), and Life Sci., Univ. Pittsburgh, Pittsburgh, PA 15261

Striatal acetylcholine (ACh) interneurons appear to be inhibited by nigrostriatal dopamine (DA) neurons. Administration of neuroleptic drugs, which block DA receptors, should result in increased ACh activity. In fact, increased striatal ACh turnover has been reported following such drugs. Both decreases in ACh levels and increases in high affinity choline (Ch) uptake have been proposed as indices of increased ACh turnover. Following neuroleptics, Ch uptake has been reported to increase (Atweh et al., Life Sci. 17:1535, 1975), while conflicting reports exist for changes in ACh levels (e.g., Trabucchi et al., Nature 249: 664, 1974; Coyle & Campochiaro, J. Neurochem. 27:673, 1976). In the present study, both Ch uptake and ACh and Ch levels were examined after treatment with the neuroleptic, fluphenazine (FLU) (2.5 mg/kg, s.c.), or saline (SAL) at pH 4.8. Male albino rats were killed by microwave irradiation, and striatum and cortex analyzed by gas chromatography. FLU decreased ACh in striatum within 15 min. The effect was maximal (-37%) by 30 min (SAL: 63.4 nmol/g; FLU: 39.9), and persisted up to 24 h. Striatal Ch levels were unchanged (SAL: 24.6; FLU: 24.2), as were cortical ACh (SAL: 14.4; FLU: 15.6) and Ch levels (SAL: 18.4; FLU: 17.8). Next, rats were decapitated 30 min after FLU or SAL, and synaptosome-rich P_2 fractions of striata prepared. Using 1 µM Ch and 37°C, no significant increase was observed in either active Ch uptake (SAL: 56.7 pmol/mg protein/2 min; FLU: 60.6) or Na^+-dependent Ch uptake (SAL: 50.1; FLU: 54.2). A coupling of high affinity Ch uptake to the rate of cholinergic nerve impulse activity has been proposed on the basis of experiments examining other brain regions. The present findings, however, suggest that, at least in the striatum, uptake of Ch is not coupled to utilization of ACh and that synthesis is not sufficiently stimulated by FLU to maintain steady-state concentrations of ACh. [Supported by NIMH grants #MH26320 (IH) and MH20620 (MJZ).]

1037 CHOLINE IN BLOOD AS A POSSIBLE INDEX OF BRAIN ACETYLCHOLINE METABOLISM IN VIVO. Tsung-Ming Shih, Ursula Kopp* and Israel Hanin. Dept. of Psychiat., Univ. of Pittsburgh Sch. of Med., Western Psychiatric Institute & Clinic, Pittsburgh, PA. 15261.

The aim of these studies has been to determine whether the rate of incorporation of deuterium-labeled choline (Ch) administered to mice in their diet may be used as an index of brain Ch and acetylcholine (ACh) metabolism. Mice were fed with Ch-[methyl-D_9]bromide (D_9-Ch) administered in drinking water as a supplement to the basic diet. Experiments with different concentrations of Ch have shown that 0.5 mg/ml will not affect normal steady-state levels of plasma Ch or brain Ch and ACh. This concentration was, therefore, used in our subsequent experiments. Fourteen days after exposure to this diet, D_9Ch was omitted from the drinking water and mice were fed with normal diets and tap water for an additional 21 days. These animals were sacrificed by microwave irradiation on days 7 and 14 during D_9Ch exposure and on days 2 and 7 following discontinuation of D_9Ch. Blood was drawn from the periorbital sinus of each mouse prior to killing. Plasma Ch and brain Ch and ACh were determined by chemical ionization gas chromatography-mass spectrometry. The data obtained are summarized in the following table:

	Days of Feeding		Days After Discontinuation of Feeding	
	7	14	2	7
Plasma Ch	3.8±0.43(12)	6.8±0.41(12)	4.2±0.13(9)	2.9±0.33(9)
Brain Ch	4.2±0.33(17)	6.2±0.35(17)	4.1±0.38(15)	2.9±0.32(16)
Brain ACh	3.8±0.27(17)	4.8±0.39(17)	3.0±0.27(15)	2.3±0.32(16)

Data are presented as mean percent ratio ± SE for the number of mice shown in parentheses. Percent ratio is calculated as the ratio of the concentration of deuterated Ch or ACh to that of the entire pool of Ch or ACh, multiplied by 100. There is an excellent correlation between plasma D_9Ch and whole brain D_9Ch and D_9ACh percent ratio during the tracer build-up stage, and the subsequent decrease in D_9Ch and D_9ACh from 0 up to 7 days following discontinuation of feeding the animals with the deuterium-labeled Ch. These findings provide further evidence that Ch pools in plasma and brain, as well as the ACh pool in brain exist in a dynamic equilibrium in vivo. The data also suggest that analyses of the metabolism and disposition of brain cholinergic components may be feasible utilizing measurements of D_9Ch mole ratios in blood of animals fed with deuterium-labeled Ch. (Supported by NIMH Grant #MH26320.)

1038 LOCAL CEREBRAL GLUCOSE UTILIZATION DURING CORTICAL SPREADING DEPRESSION IN RATS. Mami Shinohara*, Jane Jehle*, Beth Dollinger* and Louis Sokoloff. (SPON: J. Fenstermacher). Laboratory of Cerebral Metabolism, NIMH, Bethesda, Md. 20014.

Local cerebral glucose utilization (LCGU) was measured during cortical spreading depression (CSD) by the autoradiographic 2-[^{14}C]deoxyglucose technique in rats. CSD was elicited by the application of KCl crystals or filter papers soaked in 25% (w/v)KCl to the dura overlying the surface of the parietal cortex of one hemisphere (experimental side). On the opposite side (control side), filter papers soaked in normal saline were applied. LCGU studies were carried out 10 to 30 min after the first KCl application. On the experimental side, LCGU was increased in cortex to the level of 180 μmoles/100g of tissue/min, which is a higher rate of glucose consumption than in any other structures in 5 control animals while no change was produced in corresponding regions and in any other regions on the control side. The areas where LCGU was highly elevated were different in each experiment depending upon the location of trephine openings. Cortex surrounding the opening showed a very high rate of glucose consumption and deeper layers of cortex were more affected. In basal ganglia and diencephalic structures, except for hypothalamus, mamillary body, and dentate gyrus, marked decreases of LCGU were observed on the experimental side. In structures in the posterior part of the brain (vestibular nucleus, cochlear nucleus, superior olive, lateral lemniscus, inferior colliculus, superior colliculus, and cerebellum) LCGU was not changed significantly. In several studies local cerebral blood flow was measured with the iodoantipyrine method during CSD and showed high rates of blood flow in cortical regions in which glucose consumption was increased.

1039 LEAD & SEIZURES: ROLE OF γ-AMINOBUTYRIC ACID (GABA). E.K. Silbergeld, L.P. Miller*, S. Kennedy*, N. Eng* (SPON: T.N. CHASE), NIH, NINCDS, Bethesda, MD 20014

Chronic exposure of rodents to lead (Pb) from birth has been reported to alter CNS cholinergic and monoaminergic neurotransmission. These effects have been associated with hypermotility and learning disorders in animals (Neuropharm. (1975) 14, 431; Science (1975) 187, 359; Biochem. Pharm. (1975) 26, 397). Because seizures have been described in the latter stages of lead intoxication in both animals and humans, we investigated the effects of chronic Pb exposure on several aspects of the GABA system. Rats were exposed from birth to Pb acetate, 5 and 10 mg/ml in drinking water, and examined at 40-60 days of age. In four brain regions (caudate, substantia nigra, cerebellum, and parietal cortex) the following neurochemical indices were studied: GABA transaminase (GABA-T) activity, glutamic acid decarboxylase (GAD) activity, GABA levels, apparent rate of GABA synthesis (as measured by aminooxyacetic acid-induced accumulation), and synaptosomal GABA uptake and release. The results indicate that Pb exposure is associated with an increase in the apparent rate of GABA synthesis, increased GAD activity, decreased GABA-T activity, and decreased GABA levels. These changes, however, were not osberved consistently in all regions studied. The results could represent either increased GABA-mediated neurotransmission or compensatory response to an antagonism by Pb of GABA interaction at its postsynaptic receptor. This was explored pharmacologically by challenging Pb-treated rats with known convulsants. For picrotoxin and isoniazid, Pb-exposed rats had significantly lowered seizure threshholds. For pentylenetetrazole (PTZ) and strychnine, however, no differences were observed when compared to controls. These results are consistent with earlier studies on Pb-exposed mice, which showed no change in sensitivity to electroconvulsive shock, PTZ, or hyperbaric oxygen exposure. This combined neurochemical and pharmacological study suggests that the observed alterations in the GABA system represent a decrease in GABA-mediated neurotransmission which is associated with changes in the response to convulsants. Thus, decreased GABAergic function may be involved in the incidence of seizures in clinical Pb poisoning.

1040 MECHANISMS REGULATING 6-PHOSPHOGLUCONATE DEHYDROGENASE ACTIVITY IN AXOTOMIZED SUPERIOR CERVICAL GANGLIA OF THE RAT. Dominick V. Sinicropi* and Frederick C. Kauffman and Tong H. Joh. Dept. Pharmacology and Expt. Ther., School of Medicine, Univ. of Maryland, Baltimore, Md. 21201 and Lab. Neurobiol., Dept. Neurol., Cornell Univ. Med. Coll., New York 10021.

Transection of major postganglionic nerve trunks of the superior cervical ganglion elicits a reversible increase in neuronal activities of 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase (Härkönen, M. and Kauffman, F.C., Brain Res. 65:141, 1974). Elevation of these activities may be of critical importance to regeneration of injured axons since precursors used in lipid and RNA biosynthesis are formed from hexose metabolized via the oxidative pentose phosphate pathway. To study molecular mechanisms mediating the increased activity of 6-phosphogluconate dehydrogenase in axotomized superior cervical ganglia, an immunochemical assay for this enzyme was developed. The enzyme was purified approximately 1500-fold from rat brain by conventional salt precipitation, ion exchange and affinity chromatography. Two forms of 6-phosphogluconate dehydrogenase in the purified fraction were resolved by polyacrylamide electrophoresis and gel filtration; these forms are a tetramer of molecular weight $\geq$ 100,000 and a larger aggregate of identical charge:mass ratio. Separate antisera to each form of the enzyme were prepared and a microimmunochemical technique for the titration of 6-phosphogluconate dehydrogenase molecules in homogenates of single superior cervical ganglia or lyophilized sections of neural tissue weighing between 0.5 and 5 μg was developed. Antisera against each form of the enzyme cross-reacted with the other. Immunochemical titrations of axotomized and contralateral unoperated control ganglia indicated that the increase in activity produced by axotomy is due, in part, to an increase in the absolute amount of enzyme protein. Since available data suggest that immunotitration curves from the two groups are not parallel, additional mechanisms such as a change in the tetramer-polymer equilibrium or conversion of an inactive pro-enzyme to an active form may contribute to the increased activity produced by nerve transection. (Supported in part by funds from the Pangborn Foundation, University of Maryland)

1041 CALCIUM ACTIONS AND CALCIUM-BINDING PROTEIN IN ELECTROPLAX. Ari Sitaramayya*, James A. Campbell, and Frank L. Siegel. (SPON: H.A. Kubinski). Departments of Pediatrics and Physiological Chemistry, University of Wisconsin Center for Health Sciences, Madison, WI 53705.

Calcium is known to regulate several aspects of neural function, including the release of neurotransmitters from vesicular stores and the synthesis and metabolism of cyclic nucleotides, which may mediate the post-synaptic actions of some neurotransmitters. Evidence from several laboratories has shown that the calcium activation of brain cyclic nucleotide phosphodiesterase and adenylate cyclase is mediated by a calcium-dependent regulator protein (CDR). This protein is found in electroplax of Electrophorus electricus at much higher levels than in mammalian brain, the second richest source, and we have investigated its actions in this cholinergic tissue. Electroplax PDE is not inhibited by EGTA under conditions where the brain enzyme is inhibited by 50%, indicating that the major activity of PDE in electroplax is not activated by calcium and CDR. Electroplax PDE was separated into two fractions of approximately equal activity by sucrose gradient centrifugation. The first PDE activity is a cyclic AMP-specific PDE, while peak II displays activity directed against both cyclic AMP and cyclic GMP. Neither activity is inhibited by the addition of EDTA, or activated by the addition of exogenous calcium and CDR. These data indicate that CDR, the major soluble protein of electroplax, has different functions in this tissue than in mammalian brain. The actions of calcium on cyclic nucleotide synthesis and protein phosphorylation have been compared in electroplax with a high CDR content (Electrophorus) and electroplax with a lower CDR content (Torpedo). Adenylate cyclase in electroplax was stimulated 370% by 100 μM calcium. Protein phosphorylation in electroplax from Electrophorus was activated by calcium to a greater extent than that of Torpedo. Phosphorylation in Electrophorus was calcium-activated at all concentrations of ATP tested, whereas in Torpedo calcium inhibited phosphorylation at high ATP concentrations and activated phosphorylation at ATP concentrations below 100 μM. In the absence of calcium, proteins of Electrophorus electroplax incorporated ^{32}P from ^{32}P-γATP at a rate of 1 nmole/min/mg protein, while the corresponding value in Torpedo proteins is 0.2 nmole/min/mg protein; calcium exaggerates this difference. Polyacrylamide gradient gel electrophoresis reveals major differences in the membrane protein composition of the two electroplax membranes, and suggests caution in generalizing the results found in any single electroplax preparation as reflecting events at cholinergic synapses. (Supported by PHS Grant NS 11652).

1042 SIMULTANEOUS DETERMINATION OF SEROTONIN, NOREPINEPHRINE AND DOPAMINE TURNOVER IN THE TELENCEPHALON, DIENCEPHALON, MESENCEPHALON AND PONS-MEDULLA OBLONGATA OF RAT BRAIN. J.E. Smith, C. Co* and J.D. Lane*. Dept. Psychiat. and Pharmacol., Sch. Med. LSU, Shreveport, LA 71130.

Two different general approaches have been used to assess the rate of synthesis of brain biogenic amines. One method involves the use of pharmacological agents that block the synthesis of one or more of these neurotransmitters with a resulting decrease in brain content which is used to compute turnover. This method cannot be used to measure turnover in behaving animals because of the debilitating behavioral effects of the blocking agents themselves. The second general approach is to administer radioactive precursors and determine the specific activities of the biogenic amines. The intravenous administration of such precursors usually involves stressful conditions or anesthesia which can result in changes from normal in vivo synthesis rates. We report a method for measuring the concurrent turnover of serotonin (5-HT), norepinephrine (NE) and dopamine (DA) in small brain regions using a procedure for intravenous injection of radioactive precursors that can be used in awake-behaving animals without disrupting stimulus or schedule control. Six rats were implanted with chronic jugular catheters. One week later 100 µl intravenous injections of heparinized saline containing 0.5 mCi of ^{3}H-L-tryptophan and 0.5 mCi of ^{3}H-L-tyrosine were given through the jugular catheters. Sixty and 90 min later the rats were sacrificed by near-freezing in liquid nitrogen and the brains removed and dissected at -4°C into telencephalon (TEL), diencephalon (DIEN), mesencephalon (MES) and pons-medulla oblongata (P-M). The brain parts were individually pulverized in liquid nitrogen and the biogenic amines extracted into 1N formic acid-acetone (v/v-15:85). DA, NE and 5-HT were extracted using a cation exchange resin and assayed fluorometrically with compound specific micro assays that have been previously described (Smith et al., Anal. Biochem. 64, 149, 1975). A fraction of the extract was passed through an alumina column which absorbed the NE and DA but not the 5-HT. The NE and DA were eluted with acid, dried down and separated by TLC. The separate 5-HT, NE and DA fractions were assayed for radioactivity and specific activity and turnover rates determined. The highest turnover rate for 5-HT was seen in the MES (2.8 nmoles/g-hr) and the lowest in the DIEN (0.6 nmoles/g-hr). NE turnover was highest in the P-M (2.5 nmoles/g-hr) and lowest in the DIEN (0.7 nmoles/g-hr). DA turnover rate was highest in the TEL (3.3 nmoles/g-hr and lowest in the P-M (0.36 nmoles/g-hr). (This project was supported in part by USPHS Grant DA-01519-01).

1043 EFFECT OF ANOXIA ON ^{3}H 2-DEOXY-D-(^{3}H) GLUCOSE UPTAKE IN ISOLATED CEREBRAL CAPILLARIES. M. Spatz, D. Micic* and I. Klatzo. Lab. Neuropath. & Neuroanat. Sci., NIH, Bethesda, MD 20014.

A decreased 2-deoxy-D-(^{3}H)-glucose ([^{3}H] 2-DG) cerebral capillary uptake was observed in the capillary fraction isolated from brains of gerbils subjected to bilateral cerebral ischemia (Brain Res. 120, 141, 1977). In order to clarify some aspects of the pathophysiological mechanism responsible for this phenomenon, we investigated the effect of O_2 deprivation and the [^{3}H] 2-DG uptake in the cerebral capillary fraction separated from the nonvascular normal brain tissue.

The procedures for the capillary isolated and [^{3}H] 2-DG uptake studies were the same as the ones described previously (Brain Res. 110, 361, 1976) except for the different incubation solutions used in the atmosphere of various gas mixtures.

A markedly reduced (75%) specific [^{3}H] 2-DG uptake was found in the capillaries incubated in nitrogen atmosphere as compared with the one exposed to normal, or 100% oxygen or 95% N_2 5% O_2. The decreased capillary [^{3}H] 2-DG uptake was recoverable by substituting the atmosphere with normal air or oxygen after 7.5 - 15 minutes exposure to the N_2 gas. The anoxic inhibition of [^{3}H] 2-DG uptake couldn't be restored by addition of either energy phosphate metabolites or mono-, bivalent ions or various free fatty acids to the incubation buffer. Only the addition of essentially fatty acid free serum albumin in 1% concentration to the incubation mixture prevented the reduction of [^{3}H] 2-DG capillary uptake under anaerobic conditions. This process was partly abolished by the incubation mixture containing the same type of albumin with (one or more) free fatty acids.

These results suggest that the oxygen dependent [^{3}H] 2-DG uptake of cerebral capillaries may be closely related to the metabolism of fatty acids.

1044 THE BIPHASIC RESPONSE OF SYNAPTOSOMAL ($Na^+ + K^+$)-ATPase TO ETHANOL. Albert Y. Sun* and Sally L. Seaman. Sinclair Comparative Medicine Research Farm, University of Missouri, Columbia, MO 65201.

We have previously demonstrated that many membrane transport processes in the brain were altered due to the hydrophobic interaction of alcohol with membranes. High concentrations (1-5%) of ethanol inhibit synaptosomal ($Na^+ + K^+$)-activated ATPase. The inhibitory potencies of different molecular size of alcohols correlated well with their lipid solubility properties. Results of recent investigations further showed that at low concentrations of ethanol (0.1-0.5%), the ($Na^+ + K^+$)-ATPase activity of synaptic plasma membranes was enhanced. This biphasic response of enzymic activity to increasing concentrations of ethanol was also observed with other membrane transport systems such as Ca^{2+}-transport and catecholamine-uptake. The enhancement of the biochemical activities in the presence of low concentrations of ethanol is probably due to an increase in membrane fluidity. However, higher concentrations of ethanol may disturb the fine arrangements of membrane macromolecules as a result of the hydrophobic alcohol-membrane interaction. The biphasic phenomenon may explain some of the behavioral responses observed after different doses of alcohol administered. (Supported in part by USPHS Grant #AA02054).

1045 EFFECT OF CALCIUM IONOPHORES ON THE UPTAKE AND RELEASE OF CHOLINE AND ACETYLCHOLINE IN RAT STRIATAL SYNAPTOSOMES. John R. Tencati* and Roger N. Rosenberg (SPON: C.E. Spooner). Dept. of Chem., Univ. of Calif., San Diego, La Jolla, Ca. 92093.

The calcium ionophores A23187 and X537A have been used to study the effects of calcium ions and metabolic inhibitors on the high-affinity uptake of choline and the release of retained labelled choline and acetylcholine (ACh) in rat striatal synaptosomes. The sodium-dependent uptake of (^{3}H)choline was decreased in a concentration-dependent manner as the ionophore concentrations were varied from 0.10 to 5.0µM. Kinetic analysis revealed a decreased initial velocity of choline uptake and a decreased V_{max}, while the K_t was unaffected. Both ionophores were also capable of releasing choline and ACh from synaptosomes which had been preloaded with (^{3}H)choline at concentrations where high-affinity uptake prevailed (0.50µM). Release was a function of ionophore concentration, media calcium concentration, and synaptosomal calcium content. However, release alone was not sufficient to account for the low level of choline accumulated when an ionophore was present during the 30 minute synaptosome loading step.

Release of retained labelled choline and ACh from synaptosomes by A23187 was: a) partially antagonized by media calcium if the (^{3}H)choline loading step had been performed in calcium-containing media (1.5mM), b) partially dependent on media calcium if the loading step was carried out in the presence of 1.0mM EGTA, and c) absolutely dependent on media calcium if the synaptosomes had been depleted of intracellular calcium stores by treatment with carbonyl cyanide m-chlorophenyl hydrazone (1.0µM) and EGTA (1.0mM) prior to (^{3}H)choline loading. In contrast, release by X537A, which has a less specific cation selectivity than A23187 (Pressman, Ann. Rev. Biochem. 45:501, 1976), or by 60mM KCl did not require media calcium in untreated or calcium-depleted preparations. Release of labelled choline and ACh by both ionophores was greatly augmented by pretreatment with the metabolic inhibitors iodoacetic acid (1.0mM) or potassium cyanide (1.0mM). However, larger increases were seen with X537A than with A23187.

These results suggest that the calcium-dependent release of labelled choline and ACh can occur using either free-external or stored-internal calcium pools, and that release by some agents (i.e. KCl, X537A) may occur independent of calcium ion movements or because of a more efficient release of stored calcium. These studies also demonstrate that the effects of media calcium, calcium ionophores, and metabolic inhibitors on choline uptake and release in synaptosomes can be understood in terms of synaptosomal energy requirements and the mechanisms operating to regulate free cytoplasmic calcium concentrations.

1046 A HIGHLY PURIFIED PROTEIN FACTOR (α-LATROTOXIN) FROM BLACK WIDOW SPIDER VENOM EVOKED RELEASE OF NEUROTRANSMITTERS FROM DIFFERENT TYPES OF NEURONS IN MOUSE BRAIN. Mu-Chin Tzeng* and Philip Siekevitz (SPON: C.M. Connelly). Dept. Cell Biol., Rockefeller Univ. New York, N.Y. 10021.

Black widow spider venom has a wide spectrum of activity, and at least three toxic components can be separated from the venom. The major protein component (α-latrotoxin), which was purified to homogeneity (formerly referred to as fraction B_5, Frontali et al., J. Cell Biol. 68, 462 (1976)) and used in this study, is a monomeric protein with MW 130,000 and has no phospholipase activity. It causes both a marked increase in mEPP frequency and ultimate depletion of synaptic vesicles at both frog and mouse cholinergic neuromuscular junctions, but has no effect on those invertebrate systems examined. In this study, the effect of α-latrotoxin on the release of neurotransmitters from mouse brain was investigated. Mouse cerebral cortex slices were preincubated with radioactive choline, NE or GABA in oxygenated medium, and after washing with fresh medium, radioactive transmitters released into the medium were measured. Eserine, pargyline, and (aminooxy)-acetic acid were present throughout to prevent the metabolic conversion of ACh, NE and GABA, respectively. ^{3}H-ACh was determined by use of choline kinase. Since ^{3}H-NE and ^{3}H-GABA were presumably only slightly metabolized, the radioactivity in the medium, assumed to represent unmetabolized transmitters, was directly determined in both cases. In all three cases studied, α-latrotoxin markedly increased the efflux of transmitters with a latency shorter than 5 min, reaching maximum at about 10 min, and lasting for at least 30 min. At the end of the experiment, 88% of the total ^{3}H-ACh was found in the medium, and this accounted for 23% of the radioactivity originally present in the tissue. The released ^{3}H-NE and ^{3}H-GABA each accounted for ∿ 30% of that originally in the tissue. The maximal increase in magnitude of release above the base-line release was ten-fold for ACh and three-fold for NE and GABA. These effects were also observed in a Ca^{++}-free solution containing EGTA. This finding is in contrast to the transmitter-releasing β-bungarotoxin which requires Ca^{++} and has phospholipase activity. Summarizing all existing data, α-latrotoxin appears to show no selectivity towards different neuronal types of the same animal, and seems to act only on vertebrates. The effect of α-latrotoxin is probably not due to membrane depolarization, for agents such as K^+ and veratridine, which depolarize the membrane, require Ca^{++} for their effect in enhancing transmitter release. However, preliminary work (A. Gorio, M.-C Tzeng, W.P. Hurlbut, P. Siekevitz) using ^{125}I-α-latrotoxin, indicates that the toxin acts at the pre-synaptic plasma membrane.

1047 TWO MECHANISMS OF SPREADING DEPRESSION IN THE CHICKEN RETINA. A. Van Harreveld. California Institute of Technology, Pasadena, California 91125.

Two mechanisms underlying spreading depression (SD) have been proposed, a release of K (Grafstein, J. Neurophysiol. 19, 154, 1956) or a release of glutamate (Van Harreveld, J. Neurochem. 3, 300, 1959) from the intracellular compartment. Both K and glutamate cause a change in the transparency of the isolated chicken retina, with threshold concentrations of 5 mM and 0.2 mM respectively. The glutamate reaction is prevented by Mg 10-15 m equiv), the K response is not affected (Van Harreveld and Fifkova, J. Neurobiol. 4, 375, 1973). SD in the chicken retina is characterized by a change in tissue transparency and is prevented by Mg. It was therefore postulated that glutamate is involved in the mechanism of SD. However, Mg prevents SD only at relatively elevated temperatures (above 23°) At 22° or lower Mg loses its protective action. At these low temperatures the metabolism may not be sufficient to maintain the normal ion distribution of the tissue, resulting in a leakage of K ions into the extracellular space. It seems possible that under these conditions K ions are involved in SD. This is supported by the observation that ouabain (0.05 mM), which inhibits the ion pump maintaining the normal ion distribution, causes SD at elevated temperatures (32°) in the presence of Mg Also the inhibition of glycolysis, which seems to be the main energy source of the isolated retina, by iodoacetate or lack of glucose in the medium results in SD at elevated temperatures in the presence of Mg. It would seem that SD can be caused by two mechanisms, one based on a release of glutamate from the intracellular compartment, the other on a K release. There are slight differences between these forms of SD. At 22° the glutamate based SD moved at a mean velocity of 2.3 mM/min over the retina, the K based SD at 1.3 mM. The slow potential change accompanying the glutamate based SD was about twice as large as that of the K based SD. These differences were statistically significant.

The two forms of SD can be identified by the effect of Mg which prevents the glutamate based SD but not the K based one. Since the SD in the rat cerebral cortex is inhibited by Mg (Bureš, Physiologia Bohemoslov. 9, 202, 1960) it can be surmised to be a glutamate based SD. It is possible that in other tissues and under other circumstances K is involved in the propagation of SD.

1048 EFFECTS OF d- AND l-AMPHETAMINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. Lawrence R. Wechsler*, Helen Savaki*, Charles Kennedy, and Louis Sokoloff. National Institute of Mental Health, Bethesda, Md. 20014.

The actions of the potent sympathomimetic amine, amphetamine, in the central nervous system are generally attributed to its stimulation of catecholaminergic synaptic function resulting from its known influences on the release and uptake of catecholamine neurotransmitters, particularly dopamine. The [^{14}C]deoxyglucose technique provides a means to determine simultaneously the rates of glucose utilization in all the macroscopic structural components of the brain. Because of the close correlation between functional activity and energy metabolism, the method serves also to map regions of altered functional activity in the central nervous system. The [^{14}C]deoxyglucose technique has, therefore, been employed to examine the effects of d- and l-amphetamine in 40 gray and white structures of the brain of normal conscious rats. Local cerebral glucose utilization was measured during a 45-minute period beginning 15 min after the intravenous administration of 5 mg/kg of d- or l-amphetamine. d-Amphetamine caused local increases in glucose utilization in many structures of the extrapyramidal motor system as well as other structures known to contain dopamine-producing or dopamine-receptive cells. The greatest increases produced by d-amphetamine occurred in the subthalamic nucleus and the zona reticulata of the substantia nigra. l-Amphetamine causes increases in some but not all of these same structures. Decreases of cerebral glucose utilization following administration of either d- or l-amphetamine were found in the habenula and suprachiasmatic nucleus of the hypothalamus. These results indicate that amphetamine may influence behavior through effects on specific regions of the brain. Only some of these regions have been previously considered as possible sites of action of amphetamine.

1049 ATROPINE INDUCED ALTERATIONS IN CHOLINE AND ACETYLCHOLINE IN DISCRETE REGIONS OF RAT BRAIN. Lynn Wecker and Wolf-D. Dettbarn. Dept. Pharmacology, Vanderbilt Univ. Sch. Med , Nashville, TN. 37232.

Administration of atropine to rats produced dose and time dependent alterations in levels of choline (Ch) and acetylcholine (ACh) in discrete areas of rat brain. For dose-response studies, animals were injected with 2.0-40 mg/kg atropine sulfate (ip) and sacrificed 15 minutes after drug administration by focal microwave irradiation. ACh and Ch were determined in the caudate nucleus and hypothalamus by pyrolysis gas chromatography. In the caudate, ACh levels decreased from 73 to 45 nmoles/g tissue (62% control) with 20 mg/kg atropine. Higher doses of atropine had no further effect. In contrast, ACh levels in the hypothalamus were unaltered at all doses of atropine studied Concurrent with the induced ACh decrease in the caudate, atropine(10 mg/kg) produced a maximal increase in Ch levels from 20 to 40 nmoles/g tissue. Although atropine did not affect ACh levels in the hypothalamus, Ch levels increased from 17 to 26 nmoles/g tissue (153% control) with 10 mg/kg atropine. When plotted semi-logarithmically, the atropine dose-response curves were sigmoidal, with the Ch curve exhibiting marked inhibition behavior at increased doses.

The time dependency of these alterations was determined in animals treated with 5 mg/kg atropine and sacrificed 15 to 60 minutes after drug administration. In both the caudate and hypothalamus, ACh levels decreased to 75% and 77% control, respectively, by 30 minutes and values returned to control levels 45 minutes after drug administration. In contrast, Ch levels increased 15 minutes after atropine administration to 165% control in the caudate and 130% control in the hypothalamus, and values remained at this level for the following 45 minutes.

Administration of atropine has been reported to decrease total levels of ACh in brain through presynaptic mechanisms including increased ACh synthesis, turnover and release. Furthermore, results from our laboratory have shown that atropine decreases only the bound fraction of ACh. The present data indicate that a) atropine has a differential action on ACh and Ch levels in discrete brain regions and b) Ch levels are altered by doses of atropine that do not affect total ACh levels. Therefore, alterations in Ch levels induced by atropine may be intimately related to the vesicular fraction of ACh and may possibly reflect actions on Ch uptake systems or phopholipid metabolism.

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1050 CNS TERATOLOGY OF HALOTHANE, R.C. Wiggins, J.M. Rogers,* J.M. Astrello,* G.N. Fuller,* and B.M. Rigor.* Depts. of Neurobiol. & Anat. and Anesthes., Univ. of Texas Med. Sch. Houston, TX 77025.

Pregnant Sprague-Dawley rats were exposed to 0.4% halothane gas delivered in air to enclosed cages. In the first experiment each exposure lasted 30 min. 3 times daily beginning 2 days before birth and continuing postnatally until sacrifice at 20 days of age. In the second experiment, exposures of 3x1 hour, began 11 days prior to birth and were terminated at 7 days of age. These rats were sacrificed at 23 days. On the day of sacrifice, we applied a double isotope method designed to detect altered synthesis of brain subcellular fractions during metabolic perturbation (Wiggins et al. 1976 Brain Res. 107:257). In both studies 3 experimental rats received 50 uCi of 3H-leucine (IP) and three controls, born on the same day, received 50 uCi of 14C-leucine. After 3 hrs., animals were sacrificed, a 3H-labeled and a 14C-labeled brain paired, homogenized together, and the whole brain particulate subcellular fractions prepared by ultracentrifugation. Radioactivities were measured by two channel liquid scintillation counting and data were reported as ratios of 3H and 14C disentigrations (dpm). In the first experiment isotope ratio values (3H/14C= experimental/control) were: nuclear (1.7-11.5), myelin (0.72-1.0), synaptosomal (0.88-1.1), mitochondrial (1.1-1.3), and microsomal (0.79-0.90). In this experiment, the nuclear fraction had a unique high value when compared with other subfractions which had similar values. In the second experiment ratios were: nuclear (0.80-0.85), myelin (0.63-0.66), synaptosomal (0.75-0.86), mitochondrial (0.87-0.96), and microsomal (0.83-0.92). In this case a unique low value was obtained for myelin. Brain weights of 20 halothane exposed as well as 20 control rats, including weights of dissected regions (cerebellum,cerebral cortex, hypothalamus, hippocampus, midbrain, medulla oblongata, and striatum) were statistically indistinguishable. In the absence of growth stunting, the apparent high rate of nuclear membrane synthesis in exp. #1 may result from a delay in the time of maximum cellular multiplication, so that declining synthesis in controls preceeds that in halothane exposed rats. On the basis of previous experience with altered metabolic states during development, the more straight forward interpretation - atypical hyperplasia- appears less likely. In experiment #2, the myelin data are consistent with a decline and/or delay in myelinogenesis and these results are comparable to myelin effects observed in other investigations. Furthermore hypomyelination would not result in substantial weight deficits. In each experiment, altered metabolic states were observed in the absence of gross pathology. By the design of the experiment, deviation from the characteristic isotope ratio for a given paired brain homogenate indicates metabolic inequality between experimental and control rats .

1051 INTERACTION OF β-ENDORPHIN WITH RECEPTORS OF ^{3}H-DIHYDROMORPHINE, ^{3}H-NALOXONE, and ^{3}H-MET-ENKEPHALIN IN MEMBRANE OF RAT BRAIN CORPUS STRIATUM. David T. Wong* and Jong S. Horng* (SPON: L. Lemberger). The Lilly Research Labs., Indianapolis, Ind. 46206.

The binding of three radiolabeled ligands (^{3}H-dihydromorphine, ^{3}H-naloxone and ^{3}H-met-enkephalin) to membranes from rat corpus striatum could be resolved kinetically into two components, one with a low dissociation constant (high-affinity binding) and one with a high dissociation constant (low-affinity binding). Values for these dissociation constants (K_D) were estimated from Scatchard plots. The K_D values for the high affinity binding of all three ligands were increased by β-endorphin, an analgesic peptide, present at 20 nM (see Table). The K_D value for the low-affinity binding of dihydromorphine was similarly increased by β-endorphin, whereas the low-affinity binding of met-enkephalin was less affected and the low-affinity binding of naloxone was not affected by β-endorphin. The total numbers of receptor sites for dihydromorphine, naloxone and met-enkephalin, in fmoles/mg protein were 324, 410 and 1800, respectively. The number of binding sites was not affected by β-endorphin in any case. Thus β-endorphin was a competitive inhibitor of both the high- and the low-affinity binding of dihydromorphine and of the high-affinity binding of naloxone and met-enkephalin. The β-endorphin sensitive binding sites may represent opiate agonist sites, and the β-endorphin insensitive sites that bound naloxone may represent opiate antagonist sites. Approximately 75% of the met-enkephalin receptors belonged to the low-affinity binding component which were insensitive to β-endorphin; these receptors may mediate other physiologic functions besides analgesia in the central nervous system.

Radiolabeled Ligand	*K_D Values (nM)* *Control*	*+β-Endorphin*
High-affinity binding component:		
^{3}H-Dihydromorphine	1.5	3.4
^{3}H-Naloxone	1.8	3.7
^{3}H-Met-enkephalin	5.2	15.2
Low-affinity binding component:		
^{3}H-Dihydromorphine	10.4	35.5
^{3}H-Naloxone	16.8	17.9
^{3}H-Met-enkephalin	56.3	67.4

1052 REGULATION OF CSF COMPOSITION BY THE ARACHNOID MEMBRANE. Ernest M. Wright. Dept. Physiol., Sch. Med., UCLA, Los Angeles, CA 90024.

The arachnoid membrane together with the choroid plexus forms the anatomical barrier between the blood and cerebrospinal fluid (CSF). Little is known about the relative contributions of these two membranes in the regulation of the CSF composition. Using isolated membranes from the frog brain I have measured the role of the arachnoid and the choroid plexus in the transport of amino acids, anions, organic acids, and organic bases between the CSF and blood. In vivo experiments have already established that these solutes are actively eliminated from the CSF. There was net transport of anions iodide (thiocyanate, pertechnetate and bromide) and organic acids (p-aminohippuric acid) across the plexus from CSF to blood, and this was blocked by ouabain and competitive inhibitors. Even though amino acids (e.g. glycine) and organic bases (e.g. choline) were actively accumulated within the choroidal epithelium from the CSF, there was no net transport of these solutes from CSF to blood. However, choline and glycine were actively transported out of the CSF across the arachnoid membrane, and at least for glycine the kinetics of the transport process were identical to values reported for in vivo experiments. Anions (e.g. iodide) and p-aminohippuric acid were neither accumulated in the arachnoid nor actively transported across this membrane.

It is concluded that i)the arachnoid plays an important role in the regulation of amino acid and organic bases in the CSF, ii)the choroid plexus is largely responsible for the clearance of anions and weak organic acids from the CSF, and iii)the accumulation of solutes within the isolated choroid plexuses provides little information about the importance of this epithelium in the transport of solutes between CSF and blood.

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1053 CHEMICAL CHANGES IN RABBIT SCIATIC NERVE DURING WALLERIAN DEGENERATION. Allan J. Yates and Diann K. Thompson*. Div. Neuropath., Coll. Med., Ohio State University, Columbus, Ohio, 43210, U.S.A.

Gangliosides are sialic acid containing glycosphingolipids which are present in high concentrations in cerebral cortex. They are membrane-bound and their carbohydrate moieties protrude from the cell surface where they could play a role in cell surface activities such as cell-cell interaction. Data from previous studies on rabbit sciatic nerve are consistent with the bulk of them being located in the axolemma of myelinated but not unmyelinated axons. The present study was done to investigate further their cellular localization in peripheral nerve. The left sciatic nerves of 25 adult rabbits were transected and animals killed in groups of 5 at time intervals of 3, 7, 14, 21 and 28 days following surgery. The left nerves of each group were pooled as experimental and the right nerves as the controls. Lipids were extracted by the method of Suzuki (1) and gangliosides purified and analyzed by that of MacMillan and Wherrett (2). Total ganglioside sialic acid fell from the mean control value of 91.3 micrograms per gram fresh weight to 77.2 at 3 days, and 66.6 at 7 days; then it increased to the maximum value of 118.9 at 21 days and fell slightly to 111.1 at 28 days. The total ganglioside sialic acid per nerve also increased from the mean control value of 19.6 micrograms to 32.1 and 27.5 mcg. at 21 and 28 days respectively. These are in contrast to those for total cholesterol and phospholipids which steadily decreased to 66% and 35% of their respective control values by 28 days. As a per-cent of total ganglioside sialic acid G-1 and G-2 steadily decreased from their control values of 25.0% and 17.2% to 7.3% and 3.3% respectively at 28 days. In contrast, G-5 and G-6 increased from 4.4% and 1.4% to 17.8% and 17.3% respectively in the same time period. There were no changes in G-3 or G-4. Correlating these chemical changes with known histological events in Wallerian degeneration, it is suggested that most of G-1 and G-2 are in the axon while G-5 and G-6 may be in the Schwann cell. Possible cellular locations of G-3 and G-4 are not indicated from these results.

1. Suzuki, K. (1965). J. Neurochem. 12, 629-638.
2. MacMillan, V.H. and Wherrett, J.R. (1969). J. Neurochem. 16, 1621-1624.

1054 EXTENT OF 2-DIMETHYLAMINOETHANOL (DEANOL) BIOTRANSFORMATION TO CHOLINE (Ch) AND ACETYLCHOLINE (ACh) IN BLOOD AND BRAIN: A GAS CHROMATOGRAPH/MASS SPECTROMETRIC (GC/MS) ANALYSIS.

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The ability of acutely administered deanol to serve as a precursor of brain ACh *in vivo* is still unresolved. Utilizing GC/MS we, therefore, measured the extent of biotransformation in mice of acutely administered deanol to Ch in blood and Ch and ACh in brain. These compounds were administered as their deuterium-labeled analogs: deanol-[1,2-D_4]as the p-acetamidobenzoic acid salt (D_4-deanol) and Ch-[D_9-methyl]-Br (D_9-Ch). Brain, plasma and RBC levels of the nonlabeled and deuterium-labeled deanol, Ch and ACh were measured by GC/MS according to an extension of the procedure of Zahniser *et al*. (J.P.E.T. 200:545, 1977). When mice were injected intravenously with equimolar (20μmol/kg) doses of D_4-deanol or D_9-Ch, no label from the D_4-deanol, in contrast with that of the D_9-Ch treatment, was incorporated into either Ch or ACh stores in the brain up to 8 minutes post injection. Following a longer pretreatment time (30 min.) with a much larger dose of D_4-deanol (1.1mmol/kg;i.p.), plasma Ch increased with respect to control from 26.5 to 90.3 nmol/ml, while RBC Ch increased from 45.2 to 95.7 nmol/ml. The increased Ch in the plasma and RBCs was totally accounted for by D_4-Ch. Despite the elevated levels of Ch in the blood, whole brain Ch and ACh levels did not differ from those of control animals (control: ACh 24.7±0.7 nmol/gm, Ch 28.4±1.4; treated: ACh 26.1±0.6, Ch 24.2±4.1). Furthermore, while the brain concentration of D_4-deanol (87.4±8.6 nmol/gm) was extremely high, there was little evidence of any significant conversion of D_4-deanol to either D_4-Ch (2.8±4.5) or D_4-ACh (0.0). The results of these studies demonstrate that: (1) Exogenously administered deanol rapidly elevates plasma and RBC Ch levels. This effect is presumably due to conversion of deanol to Ch in the liver. (2) Increased levels of Ch in blood after deanol treatment did not induce corresponding increases in brain Ch or ACh. The D_4-Ch measured in the blood might be in an acid-soluble form (released during extraction) which does not penetrate the blood/brain barrier *in vivo*. (3) Our data do not support a direct precursor role for deanol in the synthesis of brain Ch and ACh at short times after deanol administration. They do not preclude, however, the possibility that deanol administered chronically may be a precursor of brain ACh *in vivo*.

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NEUROCYTOLOGY

1055 GEOMETRICAL CHARACTERISTICS OF THE SCHWANN CELL LAYER ENCLOSING THE SQUID GIANT AXON. W. J. Adelman, Jr., J. Moses* and R. V. Rice*. Laboratory of Biophysics, NINCDS, NIH, Woods Hole, MA 02543, Department of Biological Sciences, Mellon Institute, Carnegie-Mellon University, Pittsburgh, PA 15213, and Marine Biological Laboratory, Woods Hole, MA 02543.

To correlate series resistance, R_S, with Schwann cell layer anatomy, giant axons of *Loligo pealei* were fixed and stained. Cross and longitudinal sections were examined by transmission electron microscopy. Montage micrographs were assembled and measurements were made of the width and frequency of mesaxonal clefts at the periaxonal space and at the basilar lamina. The average mesaxonal cleft width = 10.5 nm. One cm^2 of the giant axon surface is enclosed by a single cell layer containing about 120,000 Schwann cells. One cm^2 of axon surface has a sheath mesaxonal area of 0.001 cm^2 at the periaxonal surface and 0.016 cm^2 at the basement membrane, the mesaxons branching frequently as they cross the sheath. A model was used to predict R_S. Assuming the mesaxonal clefts contain seawater, and can be lumped into a truncated cone with the narrower radius at the inner surface, then (normalized for 1 cm^2 of axon surface) $R = (\rho h)/(\pi r_1 r_2)$, where ρ = specific resistance (25Ωcm), h = width of Schwann cell layer (0.93 μm), r_1 = inner diameter of the cone (0.018 cm) and r_2 = outer diameter (0.071 cm). Thus, R_S = 0.58Ωcm^2. For an axon having 1 cm^2 of surface the volume of the Schwann cell layer = 9.55 x 10^{-5} cm^3, and the volume of the mesaxonal (extracellular) space = 6.33 x $10^{-7}cm^3$. The value of the Schwann cell membrane capacity was estimated to be approximately 0.7 μF/cm^2.

1056 GLYCOGEN AGGREGATIONS IN CENTRAL NEURONS OF INSECTS. Vincent Argiro*, Peter Pelikan*, Malcolm Wood* and Melvin J. Cohen. Dept. Biol., Yale Univ., New Haven, Conn. 06520.

In the central neurons of the cockroach Periplaneta americana, large aggregates of glycogen appear in the soma cytoplasm following prolonged exposure to CO_2 or nitrogen and also after axotomy. The large glycogen masses (1-3μm) are tightly surrounded by mitochondria and are made up of individual glycogen particles 25-50nm in diameter.

Normal thoracic ganglion neurons examined in the electron microscope show a fine scattering of individual glycogen particles in the soma. Fresh frozen 10μm cryostat sections stained with the aqueous periodic acid-Schiff procedure (PAS) show a barely discernable distribution of stained particles scattered in the peripheral cytoplasm of some large motor neurons. In approximately 5% of the large motor neurons in the normal animal stained with PAS, there is a scattering of larger stained particles in the peripheral cytoplasm.

Animals placed in a CO_2 atmosphere for 15 minutes and examined 18 hours to 5 days later show massive aggregates of PAS stained material in the peripheral cytoplasm of thoracic motor neurons. The reaction is primarily confined to the large motor nerve cell bodies. Exposure to an atmosphere of nitrogen for 10 minutes causes a similar glycogen response. The polysaccharide nature of these aggregates was confirmed by the absence of PAS staining after incubating the sections in 0.5% aqueous α-amylase.

Axotomy by amputation of a limb at the trochantor-femoral joint without anaesthesia also results in aggregates of PAS stained material on the side ipsilateral to the operation. Exposing a thoracic ganglion and cutting the nerve trunks on one side results in some cells on both sides showing the PAS positive response, with a preponderance on the operated side. The use of CO_2 was avoided in these experiments by immobilizing the animals with cold at 6°C for 5 minutes. One μm plastic sections of the axotomized preparations stained with toluidine blue shows densely stained material distributed in a pattern similar to the PAS positive material. Sections of the same cells examined in the electron microscope indicate that the aggregates stained with toluidine blue consist of 1-5μm masses of glycogen particles tightly surrounded by mitochondria.

The induction of the large glycogen aggregates by either CO_2 or nitrogen suggest that this response is caused by a lack of oxygen. Axotomy and, to a lesser extent, other procedures such as exposure of the ganglion also cause an aggregation of glycogen. The magnitude and rapidity of this glycogen response in identified insect neurons may provide a useful model for investigating factors controlling carbohydrate metabolism in central neurons.

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1057 IMMUNOLOGICAL AND BIOCHEMICAL ANALYSES OF SOME OF THE MAJOR PROTEIN COMPONENTS OF POST-SYNAPTIC DENSITIES. K. Berzins*, R.S. Cohen*, D. Grab*, and P. Siekevitz. Dept. of Cell Biol., Rockefeller University, New York, N.Y. 10021.

Post-synaptic densities (PSDs) isolated from dog cerebral cortex have been found to contain some ten major and twenty minor proteins. Actin was identified biochemically and immunologically to be one of the major proteins, though myosin was not found (Cohen et al., J. Cell Biol. 1977, in press). Some of the other major proteins have now been characterized and tentatively identified. The major component of PSDs (51,000 MW) was shown in immunodiffusion to be immunologically identical to axonal neurofilament protein. Antiserum either against purified axonal neurofilament protein or against the 51,000 MW region of PSDs separated in SDS-PAGE both precipitated one and the same component from a crude axonal neurofilament preparation and from an extract of PSDs. Yen et al. (Neurosciences Abstr. 1976) reported a similar reaction of antiserum to axonal neurofilament protein with a PSD extract. The 51,000 MW band of PSDs occasionally appeared as two closely-spaced bands in SDS-PAGE, the upper band of which co-migrated with the axonal neurofilament protein. The 18,000 MW protein of PSDs showed several similarities to the Ca^{++}-binding muscle troponin C. It co-migrated exactly in SDS-PAGE with one component of the skeletal muscle troponin complex and with the purified troponin C-like cAMP-phosphodiesterase modulator protein from bovine brain (Watterson et al., J. Biol. Chem. 251, 4501 (1976)). In addition, the 18,000 MW region of the PSD gel bound radioactive Ca^{++}. Ca^{++} seemed to be essential for the binding of the 18,000 MW protein to the PSD, as the protein is readily extracted from the structure by means of EGTA, and could be re-bound, after removal of the EGTA, by the addition of 50 mM Ca^{++}, Mg^{++} being ineffective. By means of an antiserum to purified chicken skeletal muscle tropomyosin (Jorgensen et al., Am. J. Anat. 142, 519 (1975)) we were able to precipitate one component from a PSD extract, indicating the presence of a tropomyosin-like protein in the PSD. Furthermore, this protein may be the 31,000 MW PSD protein, since the latter co-migrated in SDS-PAGE with a purified preparation of dog skeletal muscle tropomyosin. Amino acid analyses and immunological experiments are in progress to confirm the identities of both the PSD 18,000 and 31,000 MW proteins. Experiments are being performed to ascertain whether all the major proteins of myofibrils, except for myosin, are present in PSDs.

1058 BINDING OF CATIONIZED FERRITIN AND COLLOIDAL IRON TO SYNAPTIC ELEMENTS IN DISPERSED CELL CULTURES OF RAT CEREBELLUM. Richard W. Burry and John G. Wood. Dept. of Anat., University of Tennessee Center for the Health Sciences, Memphis, Tennessee, 38163

Electron microscopic studies have shown that negatively charged groups on cell surfaces bind to either cationized ferritin (CF) or positively charged colloidal iron (CI). CF has been shown to bind exposed surfaces of cultured neurons and also to aggregate into patches with increased incubation time (Wessells et al., Proc. Natl. Acad. Sci., U.S.A. 73:4100, 1976). We have investigated the mobility of negatively charged groups on both the appositional cell membranes of adjacent neurons and membranes of synaptic contacts. Dispersed cell cultures of 2 day old rat cerebellums, prepared as described by Lasher (Brain Res., 69:235, 1974), were used at 7 days in vitro.

The distribution of the CI over the plasma membranes of fixed neurons was uniform but sparse, with little or no CI binding found in the synaptic cleft. With cultures incubated in CF after fixation, very heavy globular labeling of exposed surfaces was found with almost no penetration of the CF between cells. Use of 0.1M NH_3Cl after fixation reduced the amount of binding to exposed surfaces and increased slightly the penetration of CF between cells. Incubation of unfixed cultures with CF showed scant but patchy binding to exposed surfaces and uniform heavy binding to appositional membranes, even those at some distance from the exposed surfaces. We interpret this pattern in the unfixed membranes to indicate lateral movement of the CF binding receptor from exposed surfaces to areas of apposition.

Binding of CF in fixed cultures to synaptic elements was only to exposed surfaces and not seen in the synaptic cleft. In cultures exposed to CF 10 sec. before fixation, label was seen around the synaptic contact but not in the cleft. Incubation for 1, 5, or 8 min. showed an increasing percent of synapses with CF penetrating into the synaptic cleft. Thus, synaptic junctions served as a brief barrier to the movement of negatively bound membrane groups. The number of synapses seen decreased to none after a 60 min. chase with media, and apparently, once a synapse was penetrated with CF bound groups it did not remain intact.

In summary, the mobility of CF particles indicates that negatively charged groups in developing neuronal membranes were mobile in the plane of the membrane with the exception of the synaptic junction where the mobility was partially restricted. In addition, the penetration of CF bound groups into the synaptic cleft appears to cause a reduction in the number of synapses.

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1059 SUPRAEPENDYMAL ELEMENTS ON THE VENTRICULAR SURFACE OF THE HAMSTER MEDIAN EMINENCE AND ORGANUM VASCULOSUM OF THE LAMINA TERMINALIS. J.P. Card* and J.A. Mitchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

Due to the similarity in structure of both the median eminence (ME) and organum vasculosum of the lamina terminalis (OVLT) as well as the hypothesized neuroendocrine role of ependyma in both of these regions, this study was undertaken to compare and characterize at the scanning (SEM) and transmission (TEM) electron microscopic levels the numerous supraependymal elements found in each of these regions. Brains from 25 adult hamsters were removed following intracardiac perfusion with Karnovsky's aldehyde fixative and processed for SEM or TEM. Following SEM examination, some specimens were prepared for correlative TEM. A single supraependymal cluster of neuronal cells and processes was consistently found on the floor of the ME, just anterior to the infundibular recess. Each cluster is remarkably consistent not only in size and location, but also in its surface morphology, intrinsic organization and distribution of associated processes. Neurons averaging 11u in diameter are characteristically found on the surface of the cluster and enclose a core of loosely arranged axons and dendrites. Dendrites are routinely observed arising from the peripheral neurons and passing into the core. The origin of axons within the core is less certain, but their vesicle filled endings are often seen forming synaptic contacts with the soma of the neuronal elements as well as numerous other processes within the core. A large number or processes associated with the base of the cluster contribute to an extensive network of neuronal processes on the floor and ventralmost aspects of the ventricular wall. The supraependymal elements on the surface of the OVLT exhibit a number of characteristics similar to those of the ME. Extensive networks of branching processes traverse the entire extent of the nonciliated ependymal surface covering the OVLT. However, the processes of this region rarely extend from a single structure as highly organized as that seen in the ME. In contrast, these processes accumulate at multiple foci in extensive entangled networks. Many times one or more supraependymal cells are present on the network surface, however these cells are much smaller (2-4μ vs. 11μ) than the neurons found on the ME. Other supraependymal cells (7μ) are visible on the OVLT which give rise to a number of the processes traversing the ventricular surface. While preliminary findings indicate these processes to be neuronal, extensive investigation at the TEM level is presently being conducted to determine conclusively the nature of the OVLT supraependymal elements. (Supported by NIH Grant No. RR-05387-13.)

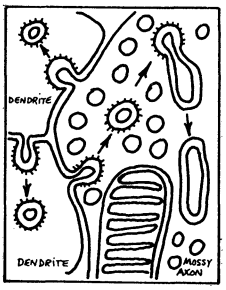
1060 PEROXIDASE UPTAKE BY PHOTORECEPTOR TERMINALS IN THE CHICK RETINA DURING DIFFERENT PHYSIOLOGICAL CONDITIONS. Nigel G.F. Cooper* and Barbara J. McLaughlin. Dept. of Anat., Univ. of Tenn. Center for the Health Sciences, Memphis, TN 38163.

Electron microscopy of in situ eyecup preparations from 1 wk. old White Leghorn chicks was used to correlate changes in photoreceptor synapses during different physiological states. Chick retinal photoreceptors are organized into three layers: an outer layer of rods and cones and two inner layers of cones (Morris and Shorey, J. Comp. Neurol., 129:313, 1967). We have used uptake of the extracellular tracer, horseradish peroxidase (HRP) and changes in the degree of dendritic invagination into the receptor terminals during light and dark adaptation as indicators of photoreceptor activity (Schacher et al., J. Cell Biol., 70:178, 1976; Schaeffer and Raviola, Symp. Quant. Biol., 40:521, 1976). In addition, magnesium (20mMg++) was added to some eyecup preparations to determine its effect on receptor synaptic activity (Ripps et al., J. Cell Biol., 70:86, 1976). When the retina is illuminated in the presence of HRP for 15-30 minutes, HRP is found in all three layers of cone terminals, in the coated vesicles, synaptic vesicles and smooth ER cisternae. The rods, filled predominantly with dense core vesicles, do not take up the tracer and coated vesicles are rarely seen. In addition,the dendrites synapsing with rod terminals are much less deeply invaginated than those synapsing with cone terminals. During similar periods of darkness, both rods and cones take up tracer, with labeled coated vesicles, ER cisternae and synaptic vesicles seen in the rods and cones. Unlabelled tubular ER becomes abundant in the cone terminals. The postsynaptic dendrites of rod terminals become much more deeply invaginated in the dark than in the light. Mg++ in the dark-adapted retina blocks the uptake of tracer in the second layer cones and their dendritic invaginations become flattened as seen in the light-adapted rods. Coated vesicles in these terminals are sparse. The other two receptor layers are unaffected by Mg++ and are similar in appearance to the normal dark-adapted state. Some tracer uptake is evident in the Mg++ treated, light-adapted retina and in all receptor terminals there is a significant reduction in the number of synaptic vesicles that is suggestive of an increased stimulation of synaptic vesicle release. In these terminals no tubular ER is present, as is found in the normal dark-adapted state, but there is an increase in the number of ER cisternae, some of which contain tracer. In summary, we have described certain anatomical changes in the photoreceptor terminals of the chick retina during light and dark adaptation and treatment with Mg++, which may indicate differences in synaptic activity between the various receptor types. Supported by USPHS Grant GM-00202 and Fight for Sight, Inc. New York City.

1061 FORMATION OF DOUBLE-WALLED COATED VESICLES IN THE DEVELOPING MOUSE CEREBELLUM: A POSSIBLE MECHANISM FOR GLOMERULAR REMODELING. Maryellen F. Eckenhoff* and J.J. Pysh. Department of Anatomy, Northwestern Univ. McGaw Medical Center, Chicago, Ill. 60611.

Double-walled coated vesicles (DWCV), consisting of an outer coated wall of invaginated mossy terminal plasma membrane and an inner wall of evaginated granule cell dendritic membrane, attached or free in the cytoplasm, were first described in electron micrographs by Andres (Z.Z.M.A. 64:63, 1964) who interpreted these structures as being components of the cyto-machinery associated with synaptic transmission. Subsequently, these structures have been interpreted in other ways.

We have noted large numbers of double-walled structures in electron micrographs of immature cerebellar mossy rosettes. DWCV's,100nm in diameter, consisted of 1) a coated invagination of mossy terminal plasma membrane enclosing an evagination of dendritic membrane or 2) coated invaginations of dendritic membrane enclosing evaginations of either mossy terminal or granule cell dendritic membrane. In the mossy terminal, these invaginations arise from either flat apposed membranes or the tips of long dendritic protrusions. Also, we have observed free DWCV's in the cytoplasm, coalescing DWCV's, and presumptive double-walled vacuoles. Cerebellar glomeruli of 16, 20, 37 and 70-day old mice were examined. All of these double-walled membranous structures were more frequent in mossy terminal cytoplasm in 16, 20 and 35-day old animals than in adults. Twenty-day old animals had the largest numbers of these structures, some 30-60 times more than adults. A serial section analysis was carried out in an attempt to elucidate the origin and fate of these structures. The results indicate that plasma membranes of adjacent cell processes are internalized and become free DWCV's that coalesce and possibly form double-walled vacuoles. However, it is unclear whether the DWCV's or vacuoles are transported retrogradely to the cell body or are disposed of in the processes. The fact that these structures are present in greatest numbers in 20-day old mossy fibers suggests they are not related to synaptogenesis, formation of desmoid junctions or synaptic transmission. We suggest that the DWCV's may be a mechanism for remodeling of the surface membranes of the cerebellar glomerulus during late development. (Supported by NIH Research Grant NS10657.)



1062 A GOLGI ANALYSIS OF THE OPOSSUM INTRALAMINAR AREA. James C. Hazlett. Dept. Anat., Sch. Med., Wayne State Univ., Detroit, MI 48201.

Previous Nissl studies of the opossum thalamus have reported three nuclei which probably constitute the entire intralaminar system in this form. From anterior to posterior they include the nucleus paracentralis (PC), the nucleus parafascicularis (PF) and the nucleus parafascicularis posterolateralis (PFP). In our Golgi preparations we have identified well impregnated long axon neurons in each of these three nuclei. Although all three cell groups contain isodendritic neurons, subtle differences in somatic size and shape, the presence of somatic spines, the dendritic arborization patterns and the relative numbers and distribution of dendritic spines permit a certain recognition of separate nuclei. However, as a consequence of dendritic overlapping, discrete boundaries between adjacent intralaminar nuclei are not always apparent. In addition, dendritic overlapping is also observed between the intralaminar nuclei and the surrounding specific thalamic cell groups. The present study, a composite reconstruction of the intralaminar area, is an attempt to demonstrate the extent and boundaries of the individual nuclei. The purposes of this approach are to (1) establish the three dimensional organization of the opossum intralaminar system and (2) to demonstrate the precise neuronal morphology necessary for the identification of separate nuclear groups. (Supported by USPHS Grant No. RR 05384-15.)

1063 OBSERVATIONS OF CELLS WHICH ENSHEATHE AXONS IN NERVE GRAFTED SPINAL CORDS. C. C. Kao* (SPON: H. E. Booker). Dept. Surgery, Sch. Med., University of Wisconsin, Madison, WI 53706.

In a transected and reanastomosed peripheral nerve, cells proliferate and form the "union scar" which unites the proximal and the distal cut ends of the nerve (J. Neurosurg. 1:400, 1944). Bared axons sprouting from the proximal cut ends of the nerve are initially ensheathed by the cells in the union scar which then become myelinated (Z. Zellforsch. 124:165, 1972).

We are reporting, in the spinal cord, structures similar to the union scar in the peripheral nerve anastomosis. The spinal cords were transected and small segments of autogenous peripheral nerve were transplanted into the spinal cord gap, oriented end-to-end with the spinal cord stumps. Within one to three weeks, cells proliferated and bridged the spinal cord-nerve graft junction.

Further electron microscopic study revealed presence of abundant bared axons at the spinal cord-nerve graft junction. These axons were swollen and contained abundant axoplasmic organelles.

Four cell types were identified in the nerve grafted spinal cord and all of the four types of cells were capable of ensheathing the axons. The cell types were (1) Schwann cells, (2) oligodendrocytes, (3) reactive glial cells, and (4) macrophages.

At a later stage, axons bridged the spinal cord-nerve graft junction and became myelinated. On the spinal cord side, the axon was myelinated by an oligodendrocyte, whereas across the junctional node of Ranvier, the same axon was myelinated by a Schwann cell.

The present observation suggests that cellular proliferation plays an important role in axonal regeneration in the nerve grafted spinal cords.

1064 ACTIN- AND MYOSIN-LIKE FILAMENTS IN BRAIN PERICYTES. Yvi J. LeBeux and Joan Willemot*. Departments of Anatomy and Biochemistry, Laval University School of Medicine, Québec, Québec, Canada G1K 7P4.

The question whether pericytes do or do not contain contractile filaments has been debated for decades. To help clarify this issue, we have investigated, using heavy meromyosin (HMM) labeling the possible actin-like nature of the 6-nm microfilaments which we observed forming bundles in the cytoplasm of brain pericytes. Pieces from rat brain were incubated in glycerol solutions of decreasing concentrations at 4° and then transferred into standard phosphate buffer (6 mM Na^+ phosphate buffer containing 100 mM KCl and 5 mM $MgCl_2$, pH 7.0) 1) with HMM, 2) without HMM, 3) with HMM+2.5 mM Na^+ pyrophosphate, and 4) with HMM+5 mM ATP. Bundles of smooth-surfaced microfilaments were seen within the pericytes of HMM-untreated nervous tissue. These filaments appeared to branch and anastomose, and to anchor on the plasma membrane. After reaction with HMM, the microfilaments were strikingly increased in number and in width to 18-20 nm, and intertwined in tightly-woven networks occupying larger areas of cytoplasm than in the pericytes from unreacted tissue. At higher magnification, their surfaces were seen to be heavily coated by short, thick side-arms cross-bridging the space between adjacent filaments at more or less regular intervals. The dense material thus observed was so tightly packed that it appeared as an irregular meshwork, where the polarity and the periodicity of the HMM-reacted filaments were distinguished with difficulty. In a few cases, typical arrowhead configurations were seen as periodic arrays of polarized elements along the surfaces of short segments of the filaments, depending upon their longitudinal orientation, or more often as single elements along the course of the filaments, interrupting the fuzzy appearance of their surfaces. When cross-sectioned, the microfilaments were seen as dense dots from which a material of lesser electron density radiated. After incubation in HMM solutions containing ATP or Na^+ pyrophosphate, they were no longer coated with thick side-arms. The microfilaments are thus of actin-like nature. In addition, after incubation in solutions containing both ATP and Mg^{++}, a drastic change occurred in pericyte cytoplasm. The smooth, 6 nm-wide microfilaments were now intermingled with, and converged onto the surfaces of, numerous, thick, tapered filaments, which we tentatively identified as being of myosin-like nature. It thus appears that certain of the major elements necessary for contraction are present in brain pericytes.

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1065 MACROPHAGES ON THE EPENDYMAL SURFACE OF THE FELINE AREA POSTREMA. R. A. Leslie and D. G. Gwyn. Dept. Anat., Dalhousie Univ. Med. Sch., Halifax, N.S., Canada B3H 4H7.

The floor of the fourth ventricle of the cat brain has been investigated with the scanning (SEM) and transmission (TEM) electron microscope. In particular, attention was directed towards supra-ependymal (SE) cells of the surface of the bilateral area postrema (AP). These cells invariably occur on the surface of the feline AP and are generally found with greater frequency on the caudal region of the organ. The surface characteristics of AP ependymal cells vary along the rostro-caudal axis of the organ, but not along the lateral-medial axis. At the rostral extreme the apical surfaces of ependymal cells of the AP are relatively smooth and flat, displaying polygonal outlines with no cilia and few microvilli. Bulbous or short filiform microvilli, generally occuring singly, are visible in this region. In contrast, the microvillus complement is more numerous and complex nearer the caudal extreme of the AP. Large tufts of microvilli, often highly-branched, are regularly seen. The tufts often occur close together and may provide a dense covering of the AP surface in the mid-to-caudal regions. SE cells interspersed with the microvillus tufts have a typical appearance suggestive of macrophages. Under the SEM they were seen to possess numerous pseudopodia-like processes. TEM revealed many large membrane-bound vacuoles distributed through out the cytoplasm of these cells. In a test for possible phagocytic activity of these cells, animals were injected intrathecally with an exogenous protein (horseradish peroxidase - HRP). Cells were later examined for the presence of HRP with the TEM after being incubated in the Graham and Karnovsky medium which produced an electron-opaque reaction product. SE cells proved to have ingested considerable quantities of HRP, most of which was contained in phagosomes. Morphologically there is great similarity between the SE cells of the feline AP and cells described as occuring regularly in the subarachnoid space of other mammals (Malloy & Low, J. Comp. Neur. 167: 257, 1976). Our findings, therefore, are strongly suggestive that macrophages regularly occur on the surface of the AP of the cat, and are generally disbursed in moderate numbers mainly on the caudal aspect of the surface of the organ.

1066 IMMUNOCYTOCHEMICAL LOCALIZATION OF GLYCEROL-3-PHOSPHATE DEHYDROGENASE IN RAT BRAIN: ARE OLIGODENDROCYTES TARGET CELLS FOR GLUCOCORTICOIDS? Paula J. Leveille*, Jean de Vellis and David S. Maxwell. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

Immunoperoxidase methods, adapted for combined light and electron microscopy, were employed to investigate the cellular localization of glycerol-3-phosphate dehydrogenase (EC 1.1.1.8; GPDH) in adult rat central nervous tissue. The use of rabbit monospecific antibodies generated against purified rat GPDH resulted in the differential staining of one glial cell population. GPDH-positive cells in perineuronal, perivascular, and interfascicular positions were identified as oligodendrocytes by classic morphological criteria.

Direct correlation of histological and ultrastructural visualization of GPDH established the cytoplasmic localization of specific reaction product within this cell type. The specificity of GPDH antigen-antibody reaction was determined by 1) Ouchterlony diffusion, 2) quantitative immunoprecipitation tests, and 3) immunocytochemical controls for both methodologic and immunologic sources of non-specific reaction product.

The illustrative data from this study defines oligodendrocytes as the structural correlate for GPDH activity in rat central nervous tissue. Quantitative evidence which demonstrates that the maximum concentration of rat brain GPDH is selectively regulated by glucocorticoids and the localization of GPDH in one cell population warrant the interpretation that oligodendrocytes are target cells for glucocorticoid activity. (Supported by USPHS Grant HD-05615 and by ERDA Contract EY-76-C-03-0012)

1067 FREEZE-FRACTURE STUDY OF SYNAPTOGENESIS IN THE CHICK OPTIC TECTUM. Catherine F. McGraw, Barbara J. McLaughlin, and Lou K. Boykins*. Dept. Anat., Univ. of Tenn. Ctr. for the Hlth. Sci., Memphis, TN. 38163.

The superficial layers of the rostral pole of the optic tectum of chicks from embryonic days (E) 6-16 and in the hatchling have been studied by freeze-fracture in order to investigate changes in the membrane structure during retinotectal synaptogenesis. Freeze-fracture analysis of early embryonic stages reveals sparsely distributed intramembrane particles on the cytoplasmic (P-face) and external (E-face) membrane leaflets of neuronal and glial plasmalemmas. Throughout development small aggregates of small-sized particles are seen on the E-faces of plasmalemmas resembling those aggregates described in freeze-fracture studies as puncta adhaerentia (Landis and Reese, J. Comp. Neur. 155: 93, 1974). By E-7, areas of loosely-arranged clusters of medium-sized particles are observed on the P-faces of presumed neurite plasmalemmas. These clusters may represent early stages of intramembrane organization at presynaptic active sites. When the underlying cytoplasm in these regions is cross-fractured, a few synaptic-like vesicles are seen which may identify the particle clusters as belonging to developing synaptic terminals. At later stages presumed vesicle fusion sites are seen interspersed with the particle clusters and complementary intramembrane specializations are observed on the E-face presynaptic membranes. Other particle aggregates are seen at early embryonic stages on the E-faces of presumed neurite plasmalemmas. They exist as solitary aggregates of large-sized particles that are closely packed. As development progresses these E-face aggregates increase in size and packing density and occupy large oval domains in the membrane. These particle aggregates are similar to the intramembrane specializations described for postsynaptic sites at excitatory synapses (Landis and Reese, J. Comp. Neur. 155: 67, 93, 1974). By E-14, there are many particle-free zones on the P-faces of cell bodies and neurites bordered by ridges of particles which appear to delineate the boundaries of neurite appositions. Many large, oval-shaped aggregates of medium-sized particles are circumscribed by these particle-free zones. Some of these P-face aggregates are seen apposed to E-faces of presynaptic regions and may represent regions complementary to the previously described postsynaptic E-face specializations or they may correspond to a second type of postsynaptic organization. In summary, intramembrane organization of particles into aggregates begins by E-7 corresponding to the time the first synapses are observed in thin sections. As development progresses these aggregates become more complex and well-defined as either pre- or postsynaptic regions. (USPHS Grants 5T01-GM00202 and RR-05423, and Fight for Sight, Inc. N.Y.C.)

1068 CLUSTERS OF BAR SHAPED PARTICLES ON INTERNODAL AXOLEMMA Rodman G. Miller, The Salk Institute for Biological Studies San Diego, Ca. 92112

Freeze fracture of the rat sciatic nerve fixed with glutaraldehyde and impregnated with 20% glycerin reveals numerous intermembranous particles on the fracture faces of internodal myelin (PNAS 72:4046). Most of these particles are small (3-7nm apparent size). Large (13nm apparent size) particles have been found on the A face of the periaxonal Schwann membrane. Many of these are arranged into hexagonal rosettes and occasionally these rosettes join sharing a pair of particles, indicating a three-fold symmetry of the particles. The adjacent, axolemmal B face, demonstrates clusters of irregularly shaped particles. Occasionally,the particle clusters are similar to the rosettes, but, more often, the irregularity of the particles obscures any symmetry that might be present (see Miller and Pinto da Silva, Brain Res., in press).

When sciatic nerve is freshly frozen (e.g., unfixed and unimpregnated with glycerin) the particles on the axolemmal B face are bar shaped. The orientation of the individual particles seems to be random with respect to the other particles in the cluster, and the length of the particles is variable and can be as long as 40nm. The morphology of the particle rosettes seen on the periaxonal Schwann A face is the same with fresh freezing and the standard fixation and impregnation procedure.

Incubation of the tissue in high calcium concentrations prior to freezing reduces the length of the bar shaped particles to that seen in fixed and impregnated tissue.

These results are consistent with a model in which the rosettes and particle clusters are connected into a single unit and the bar shaped particles are, in vivo, extended into the axoplasm. Upon facture after fresh freezing, the bar shaped particles are pulled through the inner leaflet of the axolemma and are seen upon the surface of the B face of the axon.

1069 THE PRESENCE OF SUPRAEPENDYMAL NEURONS IN THE 3rd VENTRICLE OF THE GUINEA PIG. J.A. Mitchell, D.R. Garris[1]* and J.P. Card*. Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.

Scanning (SEM) and transmission (TEM) electron microscopic studies in our laboratory have established the presence of supraependymal neurons residing within the 3rd ventricle of the hamster, rat and guinea pig. In the present study the frequency, distribution and morphology of supraependymal neurons within the 3rd ventricle of the guinea pig were investigated. Adult guinea pigs were perfused with Karnovsky's fixative and the walls and floor of the 3rd ventricle prepared for examination by correlative SEM and TEM.

Neuronal perikarya were only observed on the densely ciliated aspects of the lateral ventricular walls and were not present in all specimens examined. Considerable morphological diversity was apparent: neurons occurred singly or in densely packed clusters. Cell bodies ranged from 25-50μ in width; cell surfaces exhibited numerous protrusions and were covered by a rich meshwork of crisscrossing, varicosed fibers. Cells gave rise to multiple processes of varying diameters and lengths; processes extended for as far as 325μ over the ventricular surface. Processes either terminated on or penetrated the ependyma, or made contact with the cell bodies or processes of adjacent supraependymal neurons. Occasionally, numerous processes ran in parallel and formed large fasciculated bundles. As such bundles coursed over the ependymal surface, individual processes branched off and penetrated the subjacent ependyma. Correlative transmission electron microscopic observations indicate that both the cell bodies and processes of such supraependymal cells possess ultrastructural features characteristic of neurons. (Supported by NIH Grant No. RR-05387-13, [1]Charles B. DeVlieg Fellow.)

1070 GROWTH PROCESSES OF MATURE CORTICAL NEURONS IN FELINE GANGLIOSIDOSIS. Dominick P. Purpura,George D. Pappas and Henry J. Baker*. Dept. Neurosci. Albert Einstein Coll. Med., Bronx, N.Y. and Dept. Comp. Med., Univ. Ala., Birmingham, Ala.

Massive spine-bearing structures (meganeurites) develop at aberrant sites between the cell body and initial portion of the axon in cortical pyramidal neurons in several human ganglioside storage diseases (Purpura and Suzuki, Brain Res. 116: 1(1976). Meganeurites with extensive secondary neuritic outgrowths are also found in cortical neurons in a feline mutant with β-galactosidase deficiency and G_{M1}-ganglioside accumulation (Purpura and Baker, Nature 266:553(1977). Electron microscope studies of cortical neurons in this feline mutant indicate that outgrowths of meganeurites have morphological features of large growth processes. These features include the presence of smooth endoplasmic reticulum, occasional large,clear as well as dense core vesicles, mitochondria and vacuoles. In many instances growth processes are postsynaptic to presynaptic terminals packed with round vesicles. Apposing membranes exhibit asymmetrical densities. Growth processes arising from meganeurites may have expanded terminal heads that contain vesicles, whereas the connecting stalks have tubules and linear arrays of smooth cisternae. These growth processes are clearly distinguishable from meganeurite spine synapses. Proximal portions of meganeurites adjacent to the soma do not show surface membrane undercoating. Distal regions that merge with the axon exhibit a dense granular layer beneath the axolemma. This is suggestive evidence that the initial axonal segment is displaced distally by the formation of the meganeurite near the region of the axon hillock. The demonstration of neurites and growth processes with presynaptic endings in mature cortical neurons in ganglioside storage disease calls attention to the possible role of gangliosides in neuronal differentiation and synapse induction during cortical ontogenesis.

1071 EFFECT OF AY9944, A CHOLESTEROL BIOSYNTHESIS INHIBITOR, ON MYELIN: A COMPARATIVE STUDY BETWEEN TWO DIFFERENT PERIODS OF BRAIN DEVELOPMENT. F.A. Rawlins, F. Proverbio* and C. López-Jiménez*, Dept. Biophysics, I.V.I.C., Apartado 1827, Caracas 101, Venezuela.

The use of AY9944 [trans-1,4 bis (2-chlorobenzylaminomethyl) cyclohexane dihydrochloride], or other cholesterol biosynthesis inhibitors, opens up the opportunity to study the relative importance of the endogenous and exogenous sources of cholesterol for myelin formation and maintenance. So far, all the studies related with the effect of AY9944, a Δ^7-reductase inhibitor, on the nervous system (NS) have been carried out during the period of major brain growth spurt which is the highest vulnerable period during brain development. Therefore, the possibility exists that various of the morphological changes found in NS of animals treated within that period might be due to focal cytoplasmic degradation which is a common response of cells to various unrelated noxious stimuli. In order to clarify this point, in the present work we have carried out a comparative study of the effect of AY9944 on central NS myelin ultrastructure and chemical composition in rats treated: a) during brain growth spurt, i.e., from 1 to 21 days of age (group 1); and b) after brain growth spurt, i.e., from 30 to 70 days of age (group 2). The drug was administered every other day by intraperitoneal injection (30 mg/Kg/injection). Electron microscope (EM) analysis of group 1 revealed focal splitting and vacuolation in a large number of myelin sheaths in the optic nerves from AY9944-treated rats. Vacuolation was so pronounced that in certain areas myelin vacuoles became large electron-lucent edemas limited by 3 or 4 myelin lamellae with no continuity with any myelin sheaths. Myelin isolated from the brain of these rats was 1/3 of that from controls and 51% of its cholesterol was substituted for 7-dehydrocholesterol. The activity of $(Na^+ + K^+)$-stimulated ATPase was higher than in control myelin. EM analysis of group 2 showed no morphological difference between the optic nerve from control and AY9944-treated rats. However, isolated myelin was substituted for 7-dehydrocholesterol. Activity of $(Na^+ + K^+)$-stimulated ATPase was also higher than in control myelin. The results indicate that treatment of rats with AY9944 after the period of brain growth spurt results in the incorporation of 7-dehydrocholesterol into myelin without further changes in its ultrastructure. However, 7-dehydrocholesterol is not as efficient as cholesterol causing a slower deposition of newly formed myelin lammellae. Increment in the $(Na^+ + K^+)$-stimulated ATPase activity indicates that a functional chante is also taking place in myelin membranes of AY9944-treated rats.

1072 ABSENCE OF NODAL AND PARANODAL AXONAL MEMBRANE SPECIALIZATIONS IN FREEZE-FRACTURE REPLICAS OF JIMPY MOUSE BRAIN AND SPINAL CORD. Jack Rosenbluth. Departments of Physiology and Rehabilitation Medicine, New York University School of Medicine, New York, N.Y.

Previous freeze-fracture studies of myelinated axons in the central nervous system have revealed characteristic structures in fracture faces of the nodal and paranodal axolemma. The most distinctive of these are 1. Nearly transverse ridges on the inner leaflet of the paranodal axolemma and corresponding grooves in the outer leaflet which sometimes contain lines of particles. These ridges and grooves represent the non-junctional portion of the paranodal axolemma, which faces the helically disposed interspace between successive turns of the ensheathing glial cell. 2. An oblique pattern visible in the outer leaflet of the paranodal axolemma in the axo-glial junctional region between successive grooves. This pattern corresponds to the oblique particle rows in the inner leaflet of the glial cell membrane adjoining the paranodal axon and may also correspond to the "transverse bands" between the respective membranes. 3. Accumulations of large particles in a concentration of ~1200 per μ^2 in the outer leaflet of the nodal axolemma. It has been proposed that these correspond to the ionophores involved in the generation of the action potential at the node. In order to determine whether these characteristic nodal and paranodal features are intrinsic to the axon alone or are dependent on the presence of a surrounding myelin sheath, axons were examined from the central nervous system of Jimpy mice, which lack CNS myelin almost completely but bear normal myelin on the peripheral segments of their axons. Extensive examination of freeze-fracture replicas of the medulla and spinal cord of these animals reveals typical axons whose membrane exhibits numerous particles on the inner leaflet but very few on the outer leaflet. No examples have been found of ridges, grooves, or oblique patterns representing paranodal membrane specializations, nor have any regional accumulations of particles been seen in the outer leaflet of the axolemma corresponding to the nodal particle accumulations described previously. It is concluded on the basis of this negative evidence that the outer leaflet specialization of the axolemma at the node of Ranvier and the inner and outer leaflet specializations of the paranodal axolemma do not appear in central nervous system axons that have never been myelinated.

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1073 CORRELATIVE SCANNING/TRANSMISSION ELECTRON MICROSCOPIC ANALYSIS OF THE CEREBRAL VENTRICULAR WALLS OF PERINATAL RODENT CIRCUMVENTRICULAR ORGANS. D. E. Scott, T. H. McNeill, G. Krobisch Dudley* and W. K. Paull.* Depts. Anatomy, Univ. Rochester, Rochester, N.Y. and the Medical College of Georgia, Augusta, Ge.

The ultra-architectural organization of the developing ventricular wall of the 6 day old perinatal rat was examined with combined SEM/TEM analysis of the same tissue specimen. Tissues were prepared following the techniques of Wickham and Worthen (1973). As observed with SEM, the surface morphology of the dorsal thalamic wall was characterized by a profuse nap of microvilli and thick aggregations of knob-tipped cilia. In contrast to this typical ultrastructural appearance of the cerebral ventricular wall the surface morphology of adjacent circumventricular walls differed remarkably and in such regions as the infundibular recess and the area postrema, the dominant membranous modification of the apical surfaces of tanycytes were microvilli. Numerous supraependymal cells were noted upon the ependymal substratum. These were especially abundant in such circumventricular regions as the infundibular recess (dorsum of the median eminence) and the area postrema. Two apparent types of supraependymal cells were noted and were remarkably different in their surface organizations. One type exhibited flattened palmate processes with fluted edges and delicate filapodia. The second type of supraependymal cell possessed long primary processes that were observed to course horizontally for long distances over the ventricular surface. Subsequent analysis of previously scanned tissue with light and transmission electron microscopy revealed that ependyma which constitute the apical surface of non-circumventricular areas (such as the dorsal thalamic wall) are essentially ciliated cuboidal cells which do not penetrate the underlying parenchyma for significant distances. However, in contrast tanycytic ependyma of circumventricular organs extended processes throughout all underlying zones to terminate upon fenestrated capillaries. The ultrastructural organization of the neonatal cerebral ventricular wall is remarkably diverse and serves to suggest early functional differences from region to region in the perinatal state. The presence of two apparent classes of supraependymal cells is discussed with respect to potential histiocytic and /or neuroendocrine receptor roles that have been hypothesized in other adult mammalian species.

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1074 ORGANIZATION OF THE TEGMENTAL RETICULAR NUCLEUS IN THE ALBINO RAT. Jack C. Sipe, Joseph N. Riley and Robert Y. Moore. Dept. of Neurosciences, UCSD Sch. Med. and Veterans Administration Hospital, San Diego, CA 92037.

The tegmental reticular nucleus of von Bechterew (TRN, nucleus papilliformis) has been recognized as a prominent component of the pontine reticular formation for many years. Nevertheless, little is known of its connections or function and no previous studies of its ultrastructure have been reported. In the present investigation, the organization and synaptology of the TRN has been analyzed in material prepared for light microscopy by the Nissl and Golgi methods and by electron microscopy. Light microscopic analysis of Nissl and Golgi-stained material indicates that this nucleus, situated in a ventral paramedian position in the pontine reticular formation, is composed principally of two types of neurons. Large multipolar neurons (40-80 μ diameter) contain abundant Nissl substance and extend 4-7 sparsely branching primary dendrites up to 500 μ in length. Medium sized fusiform neurons (20-40 μ diameter) have less Nissl substance and a more closely ramifying 120-160 μ dendritic field radius. The primary dendrites of both neuronal types are usually oriented perpendicular to the long axis of the brainstem. In adult animals, very few spines are evident in the dendritic arborization of large neurons while medium sized neurons exhibit a slightly greater spine density. Ultrastructural analysis of the TRN confirms the presence of large and medium sized neurons which are compactly organized, together with numerous large dendritic trunks, between bundles of myelinated axons coursing in the rostrocaudal and transverse planes. This distinctive arrangement produces the appearance of "grey islands" between myelinated axon bundles. Within these areas a rich network of synaptic terminals virtually covers completely the available surfaces of dendrites and their perikarya. Two types of synaptic terminals are encountered in approximately equal distribution. S synapses contain 400-600 Å diameter lucent spherical vesicles and form axosomatic and axodendritic terminals with asymmetrical postsynaptic densities. F synapses are composed of flattened lucent vesicles and are distributed similar to S synapses. A variant of S and F synapses contains one or more 1000-1200 Å diameter dense granular vesicles and accounts for no more than 10% of the terminal population. Like many other sites of chemical synapse in mammalian brain, terminal boutons and boutons en passant form the majority of synaptic specializations.

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1075 EARLY STAGES OF UPTAKE AND TRANSPORT OF HORSERADISH-PEROXIDASE BY CORTICAL STRUCTURES, AND ITS USE FOR THE STUDY OF LOCAL NEURONS AND THEIR PROCESSES. H. Vanegas, H. Holländer and Hj. Distel. Max-Planck-Institut für Psychiatrie, 8000 München 40, BRD.

Horseradish-peroxidase (HRP) was injected (0.05 µl of 30% aqueous solution) into the visual cortex of adult cats, and the stages of its spread, uptake by neural structures, and retrograde descent towards the dorsal lateral geniculate nucleus (LGNd) were studied. Particular attention was given to post-injection times of 10 min to 8 hours, but animals with up to 5 days' survival were analyzed. Brains were usually processed according to Jacobson and Trojanowski (Brain Res. 74: 149; 1974).

It was found that the brown spot produced by the injection (primary diffusion) of HRP remains constant in size for about 2 hours, and then spreads out (secondary diffusion) to invade neighboring cortical areas. This invasion, however, does not result in further retrograde labelling of LGNd neurons. Local neural structures labelled during primary diffusion include a large number of infracortical axons, many of which show bifurcations directed towards the cortex, as postulated by Garey and Powell (Proc. Roy. Soc. (B) 169: 107; 1967) and Stone and Dreher (J. Neurophysiol. 36: 551; 1973). As HRP descends within these axons, fewer and deeper bifurcations appear labelled as though, after initial uptake, HRP were no longer taken up by their terminals at the cortex. Possible reasons for the lack of further HRP uptake after primary diffusion include observed local inflammatory reactions, which might also be the cause for the secondary diffusion.

Labelled neural structures include neuron somata and processes located near, but not adjacent, to the injection track. At short survival times, these are diffusely labelled with HRP and resemble Golgi-impregnated structures. At 8 hours' survival, intracellular brown granules begin to appear,and increase in number thereafter. Particularly before secondary HRP diffusion, these structures show fine details such as dendritic spine necks, terminal bouton stalks, synaptic contacts, and a remarkable degree of axonal preterminal and terminal branching. Several of these labelled structures appear alone and can be followed up with the electron-microscope. Here HRP labelling is transparent enough to allow for the characterization of, e.g., pre- and postsynaptic specializations. Labelled axons show deposits of reaction product adhering to the inner surface of the axolemma and to the neurotubuli. Diffusely labelled neurons show granular deposits in the soma and the dendrites. Labelled terminals show HRP positive deposits adhering to the synaptic vesicles and the mitochondria.

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1076 EFFECT OF DENERVATION ON THE FINE STRUCTURE AND INDOLE METABOLISM OF THE RAT AND HAMSTER PINEAL GLAND. Alan Armer. Dept. Anat., Univ. Roch., Roch., NY 14642

The influence of the pineal gland over the reproductive system is most vividly demonstrated in the golden hamster. Melatonin (MEL), an indole synthesized by the pineal of rats and hamsters, has been proposed as a mediator for this antigonadotropic effect. Whereas the control of N-acetyltransferase (NAT) activity by NE is well established in the rat, it may well be that the control of indole metabolism in the hamster is significantly different.

Animals were sacrificed 3 wks after pineal denervation by bilateral SCGx and processed for EM or utilized in organ culture (OC) studies. Denervated rat pineals display atrophic pinealocytes characterized by abundant synaptic ribbons, microtubular sheaths and lipid droplets. Dense-core (DCV) vesicles were rarely seen. Hamster pinealocytes were also atrophic. Large numbers of DCV, dense bodies and lysosomes and a sparsity of agranular reticulum were the most pronounced features of these cells.

Glands from SCGx rats and hamsters were maintained in OC in BGJb Fitton-Jackson media. 3H-5HT (48uci/um, 0.2mM) was added and a media sample obtained 24hr later. Labeled indoles were separated and quantified by TLC. During the fourth day in culture glands from SCGx rats released 13pmoles N-acetylserotonin (NAS) and 5 pmoles MEL. Similar glands stimulated with 10^{-5}M NE synthesized 180pmoles NAS and 30pmoles MEL. Under identical conditions hamster pineals released pmole amounts of NAS and MEL barely detectable by the chromatographic system. After NE there was only a slight increase in the production of NAS (6.5pmole) and MEL (6.0pmole). These data indicate that the in vitro indole metabolism of the denervated hamster pineal is significantly lower than that of the rat and is remarkably less responsive to NE stimulation.

Despite these findings, the dramatic influence which the hamster pineal exerts on the reproductive axis is abolished if its innervation is eliminated by SCGx. It may be that NE in the hamster may only partially mediate pineal NAT activity. Neural input to the hamster pineal may be more directly involved in the synthesis/secretion of a non-indolic secretory product. Such a product may be associated with the DCV found within the pineal gland of the hamster.
(Supported by NINCDS NS-11642).

1077 SEQUENTIAL POSTCASTRATIONAL CHANGES IN LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH), LUTEINIZING HORMONE (LH) AND FOLLICLE STIMULATING HORMONE (FSH). Thomas M. Badger* and Theodore J. Cicero. Dept. Psychiatry. Wash. U. Sch. Med., St. Louis, MO 63110

Castration has been used to examine certain functional aspects of the hypothalamic-pituitary-gonadal axis, particularly with respect to the regulatory role of gonadal steroids. In the course of several studies in which we attempted to use castration as a means of disinhibiting the hypothalamic-pituitary axis, we found that the level of activity in the axis was markedly influenced by the post-castration interval employed. Many investigators have examined the effects of castration on the concentrations of LH-RH and LH and FSH at selected time intervals after gonadectomy, but there have been very few studies in which changes in these hormones have been concomitantly examined at regular intervals after castration. In the present studies, we have conducted such assessments at sequential intervals from 1 to 60 days after castration. It appears that the post-castrational changes in LH-RH and LH can be divided into 2 phases. Phase 1 is characterized by an immediate 15-fold rise in serum LH levels in castrates relative to controls within 24 hours. During this phase, which lasted until day 7, there were no significant differences in pituitary LH content nor in serum or hypothalamic LH-RH levels. At about the 7th day, phase 2 began and was characterized by a marked rise in serum LH levels to a level three times the phase 1 levels by the 14th day post-castration. Coincident with this increase in LH levels, pituitary LH increased by three fold and hypothalamic LH-RH dropped to less than 50% of controls. In vitro studies indicated that the response of the pituitary to LH-RH also could be divided into two phases following castration. There were no differences in the responsiveness of the pituitary to LH-RH during phase 1, whereas during phase 2 pituitaries from castrates were more sensitive to LH-RH than were those obtained from shams. A plausible explanation for the two phases of post-castrational changes in LH-RH and LH levels may be that during the first phase changes in the levels of the releasing factor and LH reflect a release of negative feedback control, whereas during the second phase some cellular adaptations occur. Serum concentrations of FSH also increased markedly following castration, reaching a level of 3 times that of controls. Similar to LH levels, serum FSH concentrations were significantly greater in castrates than shams by 24 hours post-castration. Unlike LH, however, FSH levels continued to rise steadily until plateauing 30 days after castration. There were no significant alterations in pituitary FSH content. This research was supported in part by grants DA-01407, DA-00259 and a Research Scientist Development Award to T.J. Cicero, AA-70180.

1078 SUB-SYNAPTOSOMAL LOCALIZATION OF TRH, α-MSH, AND LHRH IN RAT HYPOTHALAMIC TISSUE. A. Barnea, G. Cho, and J. C. Porter. Cecil H. & Ida Green Ctr. for Reprod. Biol. Sci., Depts. of Ob/Gyn and Physiol., Univ. of Texas Health Sci. Ctr. at Dallas, Southwestern Med. Sch., Dallas, TX 75235.

A 10% homogenate of male rat hypothalami, prepared in 0.32 M sucrose-10 μM $CaCl_2$, was diluted either with one volume of 0.32 M sucrose-10 μM $CaCl_2$ (iso-osmotic) or with 10 μM $CaCl_2$ (hypo-osmotic). A 900 x g supernatant fluid (0.9K-S) was prepared from the diluted homogenates and fractionated on sucrose density gradients under non-equilibrium and equilibrium conditions. In the iso-osmotic 0.9K-S, TRH, α-MSH, and LHRH were each found to be sequestered in two populations of synaptosome-like particles which differed in size but were similar in density. In the hypo-osmotic 0.9K-S, the large synaptosomes containing TRH, α-MSH, and LHRH were not demonstrable; but each peptide was found to be sequestered in a single population of particles. In their sedimentation properties, the hypo-osmotically resistant particles and the small particles present in the iso-osmotic 0.9K-S were identical: (1) The banding densities of these two sets of particles under conditions of non-equilibrium sucrose gradient centrifugation (100,000 x g for 30 min) were 0.58 M for TRH and α-MSH and 0.76 M for LHRH. Under conditions of equilibrium centrifugation (150,000 x g for 180 min), the densities of the particles were 1.15 M for TRH and α-MSH and 1.25 M for LHRH. (2) The small particles present in the iso-osmotic 0.9K-S and the particles present in the hypo-osmotic 0.9K-S pelleted when centrifuged at 20,000 x g (30 min) but remained in suspension when centrifuged at 2,200 x g (30 min) (2.2K-S). If one takes as 100% the amount of peptide contained in the small particles present in the iso- or hypo-osmotic 0.9K-S, the small particles recovered from the iso- or hypo-osmotic 2.2K-S contained 80% of the TRH, 73% of the α-MSH, and 93% of the LHRH. The iso-osmotic 2.2K-S contained the small particles, but this fraction was essentially devoid of the large synaptosomes containing α-MSH and LHRH. When the iso-osmotic 2.2K-S was made hypo-osmotic by dilution with 1 vol of 10 μM $CaCl_2$ and then fractionated on the gradients, the hypo-osmotic 2.2K-S small particles were found to contain 106 ± 15.7% (mean ± SE) of the α-MSH and 56 ± 7.9% of the LHRH present in the iso-osmotic 2.2K-S small particles. If one takes as 100% the total amount of particle-bound peptides recovered after gradient centrifugation of the iso-osmotic 0.9K-S, the amount of TRH, α-MSH, and LHRH recovered in the small particles was 39 ± 1.3%, 50 ± 3.9%, and 39 ± 1.4%, respectively; the amount recovered in the hypo-osmotically resistant particles was 42 ± 1.8%, 68 ± 2.1%, and 66 ± 4.9%, respectively. It is concluded that a sub-synaptosomal pool of hypo-osmotically resistant particles containing α-MSH or LHRH is present within the large synaptosomes. This pool accounts for 36% of the α-MSH and 70% of the LHRH present in the large synaptosomes. The size of the sub-synaptosomal pool of hypo-osmotically resistant particles containing TRH is not clear.

1079 CHANGES IN BRAIN MONOAMINES ASSOCIATED WITH GROWTH ACCELERATION IN SEPTAL-LESIONED HAMSTERS. Katarina T. Borer, Michael E. Trulson and Roy A. Wise. Neuroscience Laboratory and Dept. of Psychology, University of Michigan, Ann Arbor, MI 48109, Dept. of Psychology, Princeton University, Princeton, NJ 08540, USA, and Dept. of Psychology, Concordia University, Montreal, Quebec, Canada.

Rostral septum restrains somatic growth in adult hamsters through an inhibitory influence over growth hormone (GH) and insulin release. Unilateral electrolytic destruction of rostral septum accelerates somatic growth and elevates the serum concentrations of GH and insulin (Borer, K.T. et al., Neuroend., 1977, in press).

In this study we have examined the possible involvement of monoamine neurons in the neuroendocrine control of hamster growth with two methods. First, we have measured the concentrations of serotonin (5-HT), noradrenaline (NE), and dopamine (DA) in four brain regions: hippocampus (HIP), cerebral cortex (CC), corpus striatum (ST), and diencephalon (D) in unilaterally septal-lesioned (n=6) and control (n=8) female hamsters by the method of Jacobowitz (Res. Comm. chem. Path. Pharm., 1974, 9, 29). Second, we have examined coronal and sagittal brain sections from unilaterally septal-lesioned (n=5) and control (n=4) female hamsters for evidence of NE and DA fluorescence by the glyoxylic acid method of Battenberg and Bloom (Psychopharm. Comm., 1975, 1, 3).

Eight to ten days after lesioning, during the maximal acceleration of somatic growth, there was a significant reduction in the concentrations of 5-HT in HIP (27%, $p < .001$) and CC (9%, $p < .01$) and of NE in HIP (27%, $p < .05$). Fluorescence was seen in ST (DA), locus coeruleus (NE), and septum (DA). Septal fluorescence was abolished by the lesions.

We conclude that the 5-HT projections to HIP and CC, the dorsal NE projection to HIP, and the mesolimbic DA projection to septum may be responsible for the suppression of somatic growth in adult hamsters.
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1080 EFFECT OF COMPLETE AND SELECTIVE AMYGDALOID ABLATION ON TWENTY-FOUR HOUR RESTING LEVELS OF PLASMA CORTICOSTERONE, GROWTH HORMONE, PROLACTIN, TESTOSTERONE AND HYPOTHALAMIC LHRH IN MALE RATS. Gregory M. Brown, John Chambers* and Jo A. Seggie. Neuroendocrine Research Section, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario M5T 1R8.

It has been hypothesized that the amygdala plays a role in regulating the diurnal adrenal cortico-steroid rhythm. The present study involved an examination of corticosterone and other hormonal rhythms following destructive lesions of the amygdala or of its components. Adult male rats were housed in individual cages in 3 rooms having lighting cycles of 12 hours light and 12 hours dark. Great care was taken to avoid undue disturbances to ensure that at the time of sacrifice animals were indeed in a resting state. Separate groups of rats were removed for prompt decapitation from each of the rooms to provide trunk blood samples and hypothalami at 4 hour intervals throughout the 24-hour light/dark cycle. Three separate experiments were performed involving normal controls, sham-operated controls and rats that had bilateral electrolytic lesions of the amygdala. In the first experiment, the entire amygdala complex was destroyed. In the second experiment, the cortico-medial amygdala was destroyed while in the third experiment the baso-lateral amygdala was destroyed. Destruction of the entire amygdala had no effect on normal resting levels or on the 24-hour variation of plasma corticosterone, growth hormone or prolactin. Cortico-medial amygdala destruction had no effect on normal resting levels or on the 24-hour variation of plasma corticosterone or prolactin. Destruction of the baso-lateral amygdala had no effect on normal resting levels or on the 24-hour variation of plasma corticosterone, growth hormone, prolactin or testosterone. However, preliminary data from a portion of the samples in this study suggest that the baso-lateral amygdala lesion may obliterate the normal 24-hour rhythm of hypothalamic LHRH in resting animals. Data from other studies indicate that the baso-lateral amygdala lesions also influence the adrenal stress response. On the basis of the present data, it would appear that the amygdala and its cortico-medial and baso-lateral components play no significant role in the regulation of the normal resting 24-hour hormone rhythms studied. It is suggested, however, that the baso-lateral amygdala may have a role in reproductive function and/or the ability of an animal to respond to stress.

Supported by grant #729-76/78 from the Ontario Mental Health Foundation. Dr. G.M. Brown is an O.M.H.F. Associate. Dr. Jo A. Seggie is an O.M.H.F. Scholar.

1081 PREOPTIC-HYPOTHALAMIC PERIVENTRICULAR ABLATION ALTERS ADH RELEASE AND ATTENUATES PRESSOR RESPONSES TO INTRACEREBROVENTRICULAR INJECTIONS OF ANGIOTENSIN AND HYPEROSMOTIC SOLUTIONS. James Buggy, William E. Hoffman* and A. Kim Johnson. Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Blood pressure and antidiuretic hormone (ADH) release are increased after angiotensin II(AII), hyperosmotic NaCl, or hyperosmotic sucrose solution injections into the anteroventral third ventricle of rats. Ablation of the preoptic-anterior hypothalamic periventricular tissue surrounding this region results in transient adipsia, hypernatremia, and persisting thirst deficits to systemic AII and hyperosmotic NaCl dipsogenic challenges. The following experiments on animals with this periventricular ablation examined ADH release and pressor responses to intracerebral injections of AII and hyperosmotic solutions. After electrolytic lesions aimed at the periventricular region, hydration was maintained during 1-9 days of adipsia. Lateral ventricular cannula and arterial, venous and bladder catheters were placed in sham lesioned and lesioned rats after recovery of ad lib. drinking when animals appeared in good health. Blood pressure and urine flow and conductivity were monitored in awake, unrestrained rats. An iv hydrating infusion was administered to produce a diuresis. Changes in urine conductance resulting from iv ADH injections were used to generate standard curves in each rat for estimation of ADH release. Blood pressure and urine conductance changes were then recorded after lateral ventricular injections of AII (50 and 500 ng), hyperosmotic artificial cerebrospinal fluid (10 ul of 0.5 osmol), and phenylephrine (50 ug). Blood pressure increases elicited by phenylephrine did not differ between groups whereas the pressor responses to AII and hyperosmotic stimulation were virtually abolished in lesioned rats. Analysis of ADH release to central stimulation was complicated since about 60% of lesioned rats failed to show a typical diuresis (ie., stable, low conductivity) during the hydrating infusion. In these animals, iv injection of ADH antibody lowered urine conductance suggesting incomplete ADH inhibition to the hydrating infusion. Antidiuretic responses to central stimulation were attenuated but highly variable due to the unstable baseline. The unique finding that hydrating infusions do not elicit a typical diuresis in most lesioned rats suggests inappropriate ADH release in the face of a hypotonic stimulus which normally inhibits ADH release. The data also suggest a critical role for the periventricular region in mediation of pressor responses to AII and osmotic stimuli.

1082 EFFECT OF MATERNAL DEPRIVATION ON PREWEANLING RAT SERUM GROWTH HORMONE LEVELS. S. R. Butler,* C. M. Kuhn,* and S. M. Schanberg. Dept. of Physiol. & Pharmacol., Duke Univ. Med. Center, Durham, N. C., 27710.

Our laboratory previously has reported that removal of preweanling rat pups from the mother for as little as 1 hour produces an approximately 50% reduction in brain and heart ornithine decarboxylase (ODC) activity, the first and probably rate-limiting step in polyamine biosynthesis; the decline is rapidly reversed by return to the mother (Butler and Schanberg, Trans. Am. Soc. Neurochem. 8: 259, 1977). This decrease in ODC activity is not caused by alterations in body temperature or nutritional intake, but instead appears to be due to the interruption of some mother-pup behavioral interaction. Clinically, reduced levels of serum growth hormone (GH) have been reported in maternally deprived babies and young children (D,Ercole et al., J. Pediatr. 90: 375-81, 1976). As GH given either centrally or peripherally stimulates brain ODC activity (Roger et al., Endocrinology 95: 904-11, 1974), we examined the effect of maternal deprivation on preweanling rat serum GH levels. Removal from the mother for 1, 2 or 6 hours resulted in a 40-50% reduction in pup serum GH, while return to the mother rapidly reversed this effect. GH rose to 150% of control after 15 minutes of return, and declined to baseline by 1 hour of return. These data suggest that the maternal deprivation-induced changes in preweanling rat brain ODC activity may be mediated through alterations in serum GH levels. Further, they suggest that preweanling rat maternal deprivation may be a model for the human maternal deprivation syndrome. (This research was supported by NIMH grants MH-13688 and MH-06489 and NIH grants GM-1578 and NS-05152.)

1083 LUTEINIZING HORMONE RELEASING HORMONE (LHRH) CONCENTRATION IN PITUITARY PORTAL PLASMA OF RAT: EFFECT OF CASTRATION AND SEX STEROID REPLACEMENT. Melvin Ching. Dept. Anat., Univ. Rochester Sch. Med., Rochester, NY 14642.

Sprague Dawley and Long Evans female and male rats weighing 350-450g and 450-550g respectively were left intact, castrated, or castrated and injected sc (nuchal) with 1mg testosterone proprionate (TP)/day or with 20ug estradiol benzoate (EB) 24 hours before the collection of pituitary stalk blood. Normal and castrate controls were injected with the diluent of Mazola corn oil (volume of 0.25ml). After 4 to 5 weeks the rats were anesthetized with an ip injection of 43mg α chloralose + 425mg urethane/kg BW and pituitary portal blood collected using a parapharyngeal surgical approach (Acta Endocr. 83:449, 1976). Animals were decapitated immediately following the collection of portal blood. Aliquots (100-200ul) of portal plasma and trunk plasma and whole hypothalami were extracted in 100% methanol, centrifuged, and the supernatants evaporated under N2 gas at 37°C. The residues were dissolved in Krebs ringer bicarbonate buffer, pH 7.4, for radioimmunoassay of LHRH (Neuroendocrinology 17:274, 1975). Luteinizing hormone (LH) was quantified in peripheral plasma to monitor the effect of gonadectomy and sex steroid replacement.

In both male and female castrates, plasma LH concentrations increased 14 to 16 times over normal ($p < 0.01$). Chronic TP replacement prevented the rise in plasma LH. The single injection of EB to ovariectomized rats reduced the plasma titer of LH 50% but the level of this gonadotropin remained elevated (8-fold) over normal (diestrus) ($p < 0.02$). Castration resulted in a 50% reduction in hypothalamic content of LHRH in both sexes ($p < 0.01$). Testosterone therapy elevated hypothalamic stores of LHRH to normal whereas acute EB replacement further reduced LHRH stores ($p < 0.01$). The concentration of LHRH in portal plasma did not differ from normal following ovariectomy, orchidectomy or replacement therapy (females: 172±25SE - 240±70pg/ml; males: 244±41SE - 368±80pg/ml) but was 2 to 4 times the systemic levels ($p < 0.02$ - > 0.05, 4-7 determinations/group).

The results show that castration in both male and female rats reduced hypothalamic stores of LHRH and plasma titers of LH commensurate with the magnitude of feedback by gonadal hormones without altering hypothalamic secretion rates of LHRH. Thus it appears that synthesis rather than release of LHRH is under gonadal regulation and favors the view that androgens and estrogens modulate the LHRH-induced release of LH directly at the pituitary level.

(Supported by USPHS grant HD 10912).

1084 α-MSH AND MIF-I EFFECTS ON GUANOSINE-3', 5'-CYCLIC MONOPHOSPHATE (cGMP) LEVELS IN VARIOUS RAT BRAIN AREA. C.W. Christensen*, M.A. Spirtes, C.T. Harston* and A.J. Kastin. Veterans Administration Hospital and Tulane University School of Medicine, New Orleans, LA 70146.

Alpha-melanocyte stimulating hormone (α-MSH) and a hypothalamic peptide which may inhibit the release of this hormone from the pituitary (MIF-I) exert direct effects on the brain which are expressed as changes in animal behavior. It has been suggested that these peptides may affect the cyclic nucleotides adenosine -3',5'-cyclic monophosphate (cAMP) or guanosine -3',5'-cyclic monophosphate (cGMP). Previous findings reported from our laboratory indicated that α-MSH (80 μg/kg) significantly elevated cAMP levels in the occipital cortex of both normal unoperated and hypophysectomized (hypoxed) rats (Christensen, et al., Pharm. Biochem. Behav. 5, Suppl. 1: 117-120, 1976). We now report our findings after treatment of rats with α-MSH and MIF-I followed by measurements of cyclic GMP in a number of brain areas from the same rats. Normal and hypoxed rats were administered α-MSH (80 μg/kg, daily x3) or MIF-I (1 or 10 mg/kg daily x3) in 0.9% NaCl-0.1% ascorbic acid i.p. Controls received an equivalent amount of solvent. The animals were sacrificed 30 min after the last injection in a focused microwave oven (1.5 kw; 2-3 sec). Seven different cortical and subcortical brain areas were then removed using the method of Glowinski, et al. (1966) and assayed for cyclic-GMP by the radioimmunoassay method of Steiner, et al. (1972). Administration of α-MSH did not statistically alter the cyclic-GMP level of any area of the brain in which it was measured except in the thalamus of hypoxed rats. Here it was 31% higher ($p < 0.05$) than in the controls. A smaller (17%) statistically nonsignificant rise occurred in the same area of normal rats. The lack of effect of α-MSH on cGMP levels in the occipital cortex gives added significance to our previously reported results of α-MSH elevating cAMP in this same area and its correlation with increased visual attention. Treatment with MIF-I also raised the level of cyclic-GMP in the thalamus in a statistically significant manner for both normal and hypoxed animals (21% and 63% respectively). The fact that cyclic nucleotide changes were found in both unoperated and hypoxed animals after administration of MIF-I suggests that the measured effects occurred directly in the brain without the influence of pituitary hormones. It is of interest that the "Yin-Yang" hypothesis has not been confirmed for either the occipital or the thalamic areas in these experiments. These findings are the first to indicate a direct effect of a hypothalamic peptide (MIF-I) on cGMP levels in the brain and this effect occurred in both normal and hypoxed animals. (Supported by the Medical Research Service of the Veterans Administration and NIH grant number NS 07664).

1085 EFFECT OF GLUTAMATE LESIONS OF THE ARCUATE NUCLEUS ON THE NEUROENDOCRINE SYSTEM IN RATS. James A. Clemens, Michael E. Roush* and Carl J. Shaar*. Lilly Research Labs., Indianapolis, Indiana 46206.

Lesions of the arcuate nucleus were produced by i.p. administration of 4 g/kg of monosodium glutamate to rats on days 2, 4, 6, 8 and 10 after birth, and all experimental work was performed at about 3-4 months of age. In agreement with earlier studies we noted a striking reduction in the number of cell bodies in the arcuate nucleus, a marked degeneration of the visual system and smaller anterior pituitaries, ovaries, testes and seminal vesicles in adult rats that had been treated with glutamate during the neonatal period. A 60 percent reduction in the concentration of dopamine was observed for the medial-basal hypothalamus. In glutamate-treated rats estrous cycles were irregular with several extra days of estrus, and serum levels of LH and FSH were usually lower than in control rats while prolactin levels remained unchanged. Administration of 30 mg/kg of quipazine (a serotonin agonist) or 2.5 mg/kg of chlorpromazine resulted in elevated serum prolactin levels of a magnitude similar to those seen in control animals after drug treatment. This indicates that the ability of the pituitary gland to release prolactin is not impaired after glutamate treatment. Hypothalamic content of LH-RH was found to be unchanged after glutamate treatment while PIF activity of hypothalamic extracts was slightly reduced. After ovariectomy serum LH levels were much lower in glutamate-treated rats than in control rats. In another experiment ovariectomized glutamate-treated and control rats were given 1 μg of estradiol benzoate daily for 9 days and serum levels of LH and prolactin were measured at 0800 h and 1600 h on the 10th day. Large elevations in serum LH and prolactin were observed in the control rats at 1600 h, whereas only very small elevations of prolactin and LH were found in glutamate-treated rats. In contrast, a much larger increase in serum prolactin was seen after L5-hydroxytryptophan (30 mg/kg) administration in glutamate-treated male and female rats than in control rats. These results suggest that glutamate-induced lesions of the arcuate nucleus result in an impaired release mechanism for LH and prolactin under conditions where estrogen is the "inducer", but produce a condition where serotonergic stimuli are much more effective in releasing prolactin.

1086 NOISE DETECTION AND REDUCTION BY CROSS-CORRELATION IN MULTIPLE UNIT RECORDING SYSTEMS. Donald K. Clifton*, Jamshid Rabii*, and Charles H. Sawyer. Dept. Anat. and Brain Res. Inst., Sch. Med., UCLA, Los Angeles, CA 90024

Although lacking the specificity of single unit methods, multiple unit (MU) recording techniques have the advantage of long-term stability and provide a much better indicator of local activity than the EEG. One problem with MU recording is that the signal is very small (usually < 50 μV) making the recording susceptible to noise. Electrical noise is generated within the MU system at the input stage of the amplifier and at the tissue to electrode interface, which characteristically has a high impedance.

A multiple unit recording system has been designed which eliminates amplifier and electrode noise. An electrode made of two 64 μM insulated stainless steel wires fastened together with epoxylite is placed in the area from which recordings are to be made. Each wire is attached to a standard MU preamplifier so that there are two separate MU channels measuring activity in the same area. Noise generated within the two channels will be stochastically independent; however, there is a component of the MU signal that is common to both channels. The outputs of the preamplifiers are filtered to remove the EEG and fed into an analog multiplier which, along with a low pass filter, act as a zero phase shift cross-correlator. The output of the correlator is proportional to the mean square MU activity common to both electrodes; all uncorrelated noise (i.e. electrode and amplifier noise) is cancelled out. Although biological artifacts (such as chewing) are not eliminated by this system, their presence is easily detected. This is done by correlating the signal from one wire of the MU electrode with the signal from a distant electrode. This separation precludes the possibility of there being any common MU signal, and any correlated activity would be attributable to artifact.

Construction of the system is simple and relatively inexpensive. It has been tested in rats using both acute and chronic preparations. Examples of several such tests will be presented. (Supported by grants from Eli Lilly, NIH and the Ford Foundation.)

1087 DEVELOPMENT OF THE RHESUS MONKEY THIRD VENTRICLE: SCANNING ELECTRON MICROSCOPY (SEM). P.W. Coates. Dept. Biological Structure, Univ. Wash. Sch. Med. Seattle, WA 98195.

The development of the third ventricle in a series of 5 normal fetal, neonatal and young M. mulatta monkeys was investigated with SEM. Three regions were compared: the dorsolateral wall (DW), the ventrolateral wall-floor (VF), and the choroid plexus (CP). At 132 days gestation (G132), the DW was ciliated while the VF was not. Cilia appeared shorter and more clustered compared to later ages. Surface modifications on ependymal cells of the VF, many of which are considered tanycytes, consisted of minute rounded microvilli among which were scattered longer protuberances, presumed budding cilia. Widespread polygonal surface patterns resulting from microvilli outlining cell borders, characteristic for this region in mature monkeys, were not discernible. Surfaces of the CP were relatively flat; individual ependymal cells were indistinct and covered with stubby microvilli interspersed with clusters of short cilia. By 149 days gestation (G149) the DW had prominent ridges and furrows. Cilia looked longer and less tightly clustered, obscuring underlying surfaces. Microvilli on the VF were more irregular in shape than at G132. Individual cilia and miniblebs were present. Patterning of the VF was not conspicuous. Individual ependymal cells of the CP were more distinct and possessed longer microvilli and cilia than at G132. At 12 hr post partum, although individual cells were larger, surface features resembled G149. 44 days after birth, microvilli of variable shapes covered ependymal cells on the VF, along with somewhat larger miniblebs and occasional cilia. A few unit patterns on the VF were present. Apical surfaces of ependymal CP were large and rounded. By 13 months 20 days after birth, ependyma of the VF possessed all basic surface features of the adult: microvilli, miniblebs and cilia, but did not exhibit the rich variety of detail nor extensive cell surface unit patterns. Supraependymal cells and presumed nerve processes were evident by G132 and G149, respectively, and at later ages. SEM data reveal distinct regional differences in fetal and young Rhesus monkey third ventricle. The CP and DW preceded the VF in attaining many mature characteristics shortly before and after birth. More than a year after birth, surface features and patterning of the VF, although present, were not yet elaborated to their full extent. Significant differences between males and females were not apparent. (Supported by USPHS Grant HD-10010 and by Initiative 171 Funds from the Grad. Sch., Univ. Wash.)

1088 PLASMA CORTICOSTERONE AND INSTRUMENTAL LEARNING AS MEASURES OF CLASSICALLY CONDITIONED FEAR, AND THE EFFECTS OF DEXAMETHASONE. Gary D. Coover, Betty R. Sutton*, Stephen L. Welle* and Robert P. Hart*. Dept. Psychol., Northern Illinois U., DeKalb, IL 60115.

Glucocorticoid hormones were examined as an emotional response measure and as a modulator of learning and performance. Male Long-Evans rats were first habituated to a small Plexiglas chamber with a grid floor, and then conditioned with 30 CS-US (light-footshock) pairings during a single session. On the following day they were tested in the same chamber under one of two conditions: either they were confined and presented with only the CS at 2-min intervals, or they were trained to escape the conditioned fear stimuli by jumping a hurdle into another dark chamber with a solid floor for 25 or 30 trials.

In Experiment 1 the corticosterone response to CS presentations was found to be positively related in magnitude to the intensity of footshock (.3 or 1.3 ma) used on the previous day of classical conditioning. Both footshock intensities produced larger corticosterone conditioned responses than placement in the chamber without CS or US presentations. This indicates that with this paradigm corticosterone can provide a sensitive quantitative measure of the intensity of acquired fear.

In Experiment 2, injections of the synthetic glucocorticoid dexamethasone were given 90 min prior to the classical conditioning session and again 24 hr later prior to a session of instrumental learning to escape conditioned fear stimuli. The dexamethasone caused the expected effects of decreasing the magnitude of the corticosterone response and the hurdle-jump speeds on the later blocks of trials. The effect on hurdle-jump speeds is that expected from many previous findings of a glucocorticoid facilitation of extinction of avoidance responding.

In Experiment 3, dexamethasone injections were given only prior to, or immediately following (control group), the classical conditioning sessions. Dexamethasone prior to conditioning resulted in slower hurdle-jump speeds on the early blocks of trials in hurdle-jump acquisition. However, this cannot be interpreted as an effect of deficient classical conditioning of fear, since the experimental animals attained hurdle-jump speeds equivalent to control values on the later blocks, and their corticosterone responses were not smaller during instrumental acquisition or CS presentation sessions. Two explanations for the results are that dexamethasone facilitated acquisition of a response such as freezing that competed with hurdle-jump acquisition, or that state-dependent effects were involved such that fear was not experienced on the test day until endogenous glucocorticoids were elevated.

1089 GONADAL HORMONE-CATECHOLAMINE INTERACTIONS IN DISCRETE REGIONS OF THE CENTRAL NERVOUS SYSTEM. W. R. Crowley*, T.L. O'Donohue* and D. M. Jacobowitz (SPON: Larry Ng). Lab. Clin. Sci., NIMH, Bethesda, MD 20014.

Norepinephrine (NE) and dopamine (DA) were measured in 20 discrete brain nuclei, using the punch microdissection technique and a sensitive radioenzymatic assay, and were found to vary over the estrous cycle. NE levels in the medial preoptic and paraventricular nuclei were lower on proestrus and estrus than on metestrus or diestrus. NE levels were elevated in the lateral septum and mesencephalic central gray on metestrus. Caudate DA decreased from proestrus to estrus and rose on diestrus. DA levels were elevated in the lateral septum on diestrus and in the nucleus of the diagonal band on metestrus. Median eminence DA increased from proestrus to estrus. These preliminary findings suggest that cyclic changes in catecholamines may be related to fluctuations of gonadotropins and ovarian hormones to influence ovulation and mating behavior.

In a second experiment, sex differences were found to exist in catecholamine levels in discrete brain regions in adult, intact male and female rats. Males contained more NE than females in the median eminence and in the paraventricular, periventricular, arcuate and preoptic-suprachiasmatic nuclei as adults, and more DA than females in the median eminence and caudate, arcuate and diagonal band nuclei.

Castration of males at 1 day of age tended to lower NE in the arcuate and preoptic-suprachiasmatic nuclei and significantly lowered DA in the arcuate and diagonal band nuclei to levels similar to the female. Administration of 1.25 mg testosterone propionate to females of 4 days of age significantly elevated DA in the arcuate nucleus and tended to elevate DA in the diagonal band nucleus and NE in the arcuate and preoptic-suprachiasmatic nuclei to levels similar to males. These results suggest that the catecholamine innervation of the above-cited areas is altered during sexual differentiation.

1090 BEHAVIORAL SPECIFICITY OF THE INHIBITORY EFFECT OF PROGESTERONE IN THE HAMSTER. Joseph F. DeBold*, Julie L. Morris*, and Lynwood G. Clemens* (SPON: K. L. Lovell). Dept. Zoology, Michigan State Univ., E. Lansing, MI 48823.

In addition to its well-known facilitative action, progesterone can inhibit estrogen-induced sexual receptivity. We have recently demonstrated that the female hamster is particularly sensitive to this inhibitory action of progesterone. However, the behavioral, sexual and steroidal specificity of this effect has not previously been examined. In a series of 3 experiments the possible effects of progesterone (P) were assessed on testosterone propionate (TP) and estradiol-benzoate (EB) induced male copulatory behavior in males and TP and EB induced sexual receptivity in both male and female hamsters.

Immediately following castration, male hamsters were given daily replacement therapy with 150 ug TP, 150 ug TP + 500 ug P, 5 ug EB, 5 ug EB + 500 ug P, 50 ug EB, 50 ug EB + 500 ug P, 500 ug P or the oil vehicle. In weekly tests for male copulatory behavior TP treated males performed at precastration levels, while animals receiving EB stopped ejaculating. Treatment with P did not alter the response to TP or EB, nor did P have any apparent activity when given alone. In subsequent weeks the dose of P was increased to 2.5 mg without effect. In order to maximize any possible effect of P, male hamsters were castrated, but replacement did not begin for 8 weeks. Animals receiving 150 ug TP + 2.5 mg P returned toward precastration levels at a slightly slower rate than the group receiving 150 ug TP alone. In the final experiment, male and female hamsters were gonadectomized and then 2 weeks later began daily treatment with 5 ug EB, 5 ug EB + 500 ug P, 50 ug EB, 50 ug EB + 500 ug P, 150 ug TP, 150 ug TP + 500 ug P, 500 ug TP or 500 ug TP + 500 ug P. In weekly tests for sexual receptivity, both males and females receiving chronic P exhibited greatly reduced lordosis duration. Although TP was much less effective for inducing receptivity than EB, P inhibited the response to either steroid.

Our experiments demonstrate that chronic P administration is virtually without effect on androgen or estrogen induced male copulatory behavior. However, P is capable of reducing androgen or estrogen-induced receptivity in both male and female hamsters. Thus, P appears to be behaviorally specific but not sex or steroid specific.

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1091 A SYSTEM OF GAP JUNCTIONS AND SOME UNUSUAL GLIAL-NEURONAL CONTACTS IN THE ARCUATE NUCLEUS OF ALBINO RATS. Manuel del Cerro and Karl M. Knigge. Ctr. Brain Res. and Dept. Anat., Sch. Med., U. of Roch., Rochester, N.Y. 14642.

During the course of a study on the ontogenesis of the arcuate region of the ventromedial hypothalamus we have observed numerous instances of cell-to-cell contact between glial cells and/or glial cells and neurons. These contacts may be important for the neuroendocrine functions of this site. Albino rats, aged from 20 to 60 postnatal days, were perfused with aldehydes and their hypothalami post-fixed with OsO_4 and treated in block with uranyl acetate. The latter chemical increases the electron density of the tissue and also prevents the formation of spurious cellular appositions (Brightman and Rees, 69). The material was studied simultaneously by optical and electron microscopy. An extensive system of gap junctions was observed along the somas of the tanycytes (radial glia) which line the ventricular wall and extend at great distances within the neuropil of the arcuate nucleus. Numerous gap junctions, or nexi, occur at the level of the head and neck of the tanycytes, joining them with the bodies of adjacent tanycytes or their side projections. Some of these nexi are unusually extended, sometimes surrounding the entire circumference of a tanycytic process, or involving three or more participant cells. By optical microscopy it is possible to observe frequent contacts en passant between tails of tanycytes and neuronal somas; electron microscopically it has been possible to observe gap junctions in some of those contacts. A noteworthy form of cell-to-cell contact within the arcuate nucleus is the presence of a unique synaptoid contact between the neck of tanycytes and large dendrites; these formations involve the presence of paramembranous densities, with accumulation of vesicles on the glial side. From the functional point of view these observations indicate that physical channels communicate tanycytes with each other and with neurons. The significance of the synaptoid contacts between tanycytes and dendrites requires further study.

(Partially supported by grant RR 05054)

1092 COMPARISON OF THE EFFECT OF MEDIAL FOREBRAIN BUNDLE, RAPHE OR SUPRACHIASMATIC NUCLEAR ABLATION ON THE 24-HR PERIODICITY IN PLASMA CORTICOSTERONE LEVELS. J. D. Dunn, A. J. Castro and J. A. McNulty*. Dept. Anat., Loyola Univ. of Chicago, Stritch Sch. Med., Maywood, Ill. 60153.

Monoaminergic systems have been implicated in rhythmic neuroendocrine activity and associated behavior but information regarding specific nuclei and/or pathways is still lacking. Thus the present study was undertaken to determine whether the circadian periodicity characteristic of pituitary-adrenal function is abolished after ablation of the raphe nuclei (RN), medial forebrain bundle (MFB) or suprachiasmatic nuclei (SCN). Additionally, the daily variation in body temperature was evaluated. Non-stress plasma corticosterone levels were assessed fluorometrically in blood samples obtained from a tail vein of individual adult female rats at 4 hr intervals during 24-hr and 36-hr periods, 3 mo. and 6 mo. after surgery, respectively. Body temperature was recorded every 4 hr during a 48-hr period (4 mo. post-surgery) using a tele-thermometer and rectal probe.

The highest corticosterone levels for most intact, MFB and RN animals were obtained in samples collected just prior to the period of light-dark transition. The amplitude of the rhythm in MFB but not RN rats was similar to that noted for controls; RN rats presented a "flattened" rhythm. Individual SCN rats demonstrated steroid excursions of normal amplitude but they were asynchronous and devoid of circadian rhythmicity. All groups showed circadian periodicity in body temperature; the amplitude did not vary among the groups and the highest temperatures were recorded during the latter half of the dark period.

The data indicate that neither the rhythmicity in plasma corticosterone levels nor body temperature is abolished after ablation of the ascending monoaminergic systems and that ablation of the SCN abolishes the periodicity in pituitary-adrenal function but not body temperature.

1093 EFFECTS OF HIPPOCAMPAL LESION ON BLOOD PRESSURE, PLASMA CORTICOSTERONE AND SOCIAL BEHAVIOR IN DIFFERENT ENVIRONMENTAL CONDITIONS. Daniel L. Ely, Ernest G. Greene and James P. Henry.* Dept. Biology, Univ. Akron, Akron, OH 44325, Dept. Psychol. and Dept. Physiol., Univ. So Calif., Los Angeles, CA 90007.

The aim of the present study was to investigate the role of the hippocampus upon autonomic nervous system function and social behavior in competitive and non-competitive conditions.

Hippocampal-lesioned (HL) male CBA mice competing for territory and social position for 81 days in a population cage developed systolic hypertension (160 mmHg, $P < .01$) as compared to unoperated controls (UC, 131 mmHg) or cortical lesioned controls (CLC, 134 mmHg). However, HL animals living in a non-competitive environmental condition (standard laboratory living conditions for mice) did not develop hypertension (137 mmHg) nor did they significantly differ in blood pressure from the UC (126 mmHg) or CLC (136 mmHg) groups in the same environmental conditions. Similarly, the HL animals in the competitive condition showed significantly elevated plasma corticosterone (21 ug%, $p < .05$) as compared to UC (14 ug%) or CLC (14 ug%). Whereas, the HL animals living in the non-competitive situation exhibited no significant difference in corticosterone (9 ug%) from the other control groups under the same conditions (UC-11 ug% and CLC-10 ug%).

Computer based behavioral measurements showed that the HL animals failed to develop a social hierarchy with dominant and subordinate individuals and they displayed hyperactivity with disorganized behavior patterns as compared to control groups. The HL groups in the competitive situation also failed to show aggressive responses to an intruder animal. When the roles were reversed and they were now the intruder in another colony they showed abnormal escape behavior and received 3 times more attacks than did controls.

As an alternative to unitary constructs of hippocampal function the hippocampus may have several different functions including modulation of social behavior and secondary effects on the autonomic and endocrine systems. (Research supported by NIH grants MH19441, MH17706, and MH26155).

1094 THE EFFECTS OF NEONATAL ADMINISTRATION OF LOW DOSES OF ESTRADIOL BENZOATE UPON THE DIFFERENTIATION OF SEXUAL BEHAVIOR IN THE FEMALE HAMSTER. Anne M. Etgen* and Richard E. Whalen. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Several lines of research suggest that androgens may exert their effects on sexual differentiation only after they have been enzymatically converted to estrogens. This aromatization hypothesis is supported by the demonstration that estrogens, but not non-aromatizable androgens, can effect behavioral differentiation in rodents. The normal adult female hamster is behaviorally unresponsive to androgen stimulation. However, female hamsters can be masculinized by the injection of as little as 1.0 ug of testosterone propionate (TP) at birth. This observation suggested that perhaps sexual differentiation of the hamster is mediated by androgens since somewhat less than 1 percent of testosterone is aromatized to estrogen. To test this hypothesis, the effects of very low doses of estradiol benzoate (EB) administered at birth upon the expression of sexual behavior in the adult female hamster were investigated.

Female hamsters were injected subcutaneously within 24 hours of birth with oil vehicle, 1.0 ug TP (a dose which has been shown to masculinize but not defeminize the female hamster), or with 0.05, 0.10, 0.50, 1.0, or 2.0 ug EB. All animals were ovariectomized between 55-65 days of age and tested for the occurrence of both male and female sex behavior in response to appropriate hormonal stimulation. Male behavior was measured as the average number of (a) mounts without pelvic thrusting (M) and (b) mounts accompanied by pelvic thrusting (M+T) in each of 3 weekly 10 minute tests with a receptive female. Female behavior was measured as the average total lordosis duration (TLD) in 3 weekly 10 minute tests with sexually experienced males.

All doses of EB elicited more male sexual behavior than oil vehicle, and a one-way analysis of variance indicated that 0.5 ug EB elicited significantly more Ms ($F=7.87$; $df=1,26$; $p<.01$) and M+Ts ($F=14.16$; $df=1,26$; $p<.01$) than 1.0 ug TP, and that 0.05 ug EB induced as many M+Ts as 1.0 ug TP. In addition, EB decreased the average TLD in a dose-dependent fashion when compared to vehicle-injected controls while TP had no effect on lordosis.

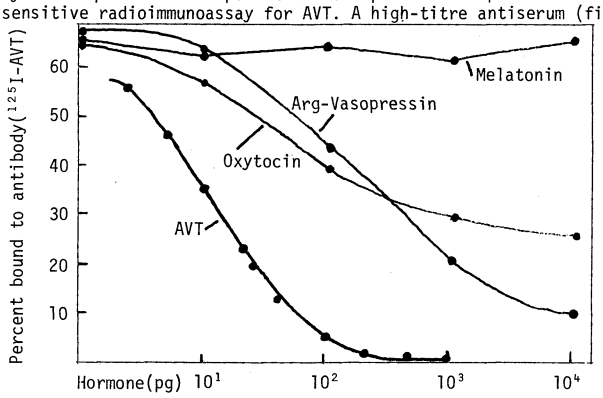
The fact that low doses of neonatally administered estrogen can both masculinize and defeminize the behavioral responses of adult female hamsters much more effectively than androgens provides additional evidence that the differentiation of rodent sexual behavior may indeed be mediated by the action of estrogens during a critical period of development. Further examination of this possibility is being conducted by determining the effects of extremely low doses of RU-2858, a potent synthetic estrogen, upon behavioral differentiation in the female hamster.

1095 THE EFFECT OF DIFFERENTIAL FORNIX ABLATIONS ON THE CIRCADIAN RHYTHMICITY OF ADRENAL CORTICOSTEROIDS. Christine Fischette*, B.R. Komisaruk, H. Edinger, H. Feder‡ A. Siegel. Depts. of Physiology and Neurosciences, College of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103, and Institute of Animal Behavior, Rutgers University, Newark, New Jersey 07102.

Meibach and Siegel (Brain Res 124: 197, 1977) found that fibers from the dorsal hippocampal formation travel in the medial part of the fornix, while fibers from the ventral hippocampal formation travel in the lateral part of the fornix. The medial corticohypothalamic tract, whose cell bodies lie in the anteroventral subiculum, travels in the most lateral part of the fornix and fimbria and terminates in the basal hypothalamus from the level of the suprachiasmatic to the arcuate nucleus. Thus, lateral or medial fornix ablations were performed on adult male rats in order to selectively ablate the medial corticohypothalamic tract. The circadian rhythmicity of corticosterone secretion was assessed 1-2 weeks postoperatively. Analysis of corticosterone was determined by the competitive protein binding method or by radioimmunoassay. On the basis of preliminary data, interruption of the lateral fornix disrupted the cyclicity of corticosterone secretion that is normally synchronized with the light-dark cycle, whereas medial fornix ablated animals or cortical controls showed no such disruption. Group mean levels of corticosterone in the lateral fornix ablated rats were intermediate between peak and nadir levels in the controls. An individual analysis of lateral fornix ablated animals, however, reveals that circadian peaks of corticosterone occurred, but were not synchronized with the light-dark zeitgeber. We speculate that the anteroventral subiculum modulates the timing of the normally-occurring circadian peak of corticosterone via the medial corticohypothalamic tract. (Supported by NINCDS Grant # NS 07941-08).

1096 DEVELOPMENT OF A NEW RADIOIMMUNOASSAY FOR ARGININE-VASOTOCIN[AVT] Laurel A. Fisher*, Bernadette M. Cusack*, and John D. Fernstrom, Laboratory of Brain and Metabolism, M.I.T., Cambridge, MA 02139.

The measurement of arginine-vasotocin, a nine amino acid peptide, in animal tissues and blood, has been limited to date to a complicated, inferrential bioassay, save for a brief period recently when an indirect radioimmunoassay method was available. The ability to study the potential biologic significance of this peptide has thus been encumbered by the unavailability of a standard assay technique. We now report the development of a specific and sensitive radioimmunoassay for AVT. A high-titre antiserum (final



dilution in assay 1:240,000) was generated in New Zealand white rabbits using a bovine thyroglobulin conjugate of AVT (Skowsky and Fisher, J.Lab.Clin.Med.80:134,1972)[synthetic AVT, 95% pure, was kindly provided by Dr. R. Hirschman, Merck, Sharp, and Dohme, Rahway, NJ]. The assay is basically a modification of the method of Skowsky et al.,J.Clin.Endocr.Metab.38:278,1974, using Norit-A to separate antibody-bound and free AVT. The sensitivity and specificity are indicated in the above figure; melatonin, oxytocin, and vasopressin levels in blood and pineal are not great enough to interfere with the quantitation of AVT in these tissues. Studies are currently in progress using this assay to determine the presence of AVT in pineal, blood, and brain, and hopefully to assess its physiologic significance.

1097 EFFECTS OF IN VIVO STIMULATION OF POSTERIOR PITUITARY ON PROTEIN CARBOXYMETHYLASE AND ITS SUBSTRATE. Claude Gagnon , and Julius Axelrod. Lab. Clin. Sci., N.I.M.H., Bethesda, Md. 20014

Protein carboxymethylase (PCM) activity has been shown to be high in brain and endocrine tissues. Diliberto et al. (Proc. Natl. Acad. Sci. USA 73: 4050-4054, 1976) demonstrated the presence of substrate for this enzyme on the membrane of chromaffin granules from adrenal medulla and suggested a possible role of this enzyme in exocytotic secretion. To investigate this possibility, rats were given 2% NaCl in their drinking water. Under these conditions the posterior pituitary is strongly stimulated and vasopressin is released by exocytosis. Posterior pituitary PCM activity and its substrate, the methyl acceptor proteins (MAP) were measured. The MAP dropped rapidly to 80, 40 and 20% of the control levels after 1, 2 and 4 days of treatment and remained at the 20% level up to 28 days. On the other hand, the PCM activity was not affected in the first week. After a lag of 2 weeks there was a significant increase in the enzyme activity. PCM activity rose 62% above control level after 4 weeks. This is the first demonstration of an enhancement of PCM activity following strong stimulation of a gland. This finding supports a role for PCM in neurosecretion.

1098 POSTERIOR MEDIAL HYPOTHALAMIC MEDIATION OF FEMALE COURTSHIP BEHAVIOR IN THE RING DOVE. Marie J. Gibson* and Mei-Fang Cheng* (SPON: K. L. Keim) Inst. of Animal Behavior, Rutgers, Newark, NJ 07102

When a female ring dove is exposed to a courting male, gonadal development occurs which has been correlated with an increase in courtship behaviors and rising titers of estrogen and progesterone (Cheng,J. Endocr. 63:43, 1974). Estrogen, which plays a role in regulating sexual crouching and nestcooing, is shown by autoradiography (Martinez-Vargas, Stumpf and Sar, J. Comp. Neur. 167: 83, 1976) to be taken up in the preoptic (POA) and posterior medial hypothalamic (PMH) regions. The purpose of this study is to examine the role of the hypothalamus in mediating estrogen dependent behaviors in the female ring dove.

In the first experiment laparotomies and behavioral tests were conducted to determine the effects of electrolytic lesions in intact females. Neither gonadal development nor courtship behavior was affected by lesions in the anterior hypothalamus or through the anterior portion of the PMH. However, lesions just rostral to the POA caused gonadal atrophy. No nestcooing and a gradual decline in sexual crouching were observed. Of lesions in the posterior region of the PMH only 1 of 4 caused gonadal atrophy, yet all courtship behaviors were abolished in 3 and greatly reduced in the 4th female.

In the second experiment, exogenous estrogen failed to restore courtship behaviors in intact females with lesions in the posterior portion of the PMH.

Third, ovariectomized females were tested daily with a male while receiving daily injections of estrogen. Behavior was compared under this regimen for a period before and after sham or electrolytic lesions. Sham lesions or those beyond the posterior PMH caused no interruption of estrogen induced sexual crouching or nestcooing, while posterior PMH lesions virtually abolished the behavioral effect of estrogen treatment.

These results suggest that the effects on courtship behavior found with anterior POA lesions are associated with interruption of gonadotropin release. However, the posterior PMH is apparently involved in the mediation of estrogen dependent courtship behaviors independent of its possible role in regulation of gonadotropins.

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1099 THE EFFECTS OF ESTROGEN ON THE SINGLE UNIT ACTIVITY OF THE HYPOTHALAMUS AND PREOPTIC AREA IN ESTROGEN-PRIMED AND NON-PRIMED OVARIECTOMIZED FEMALE RAT. D. P. Gilman,* J. C. Hitt and N. R. Remley. Dept. of Psych., TCU, Ft. Worth, TX 76129

Estrogen has been reported to have both depressive and facilitatory effects upon luteinizing hormone (LH) release in ovariectomized rats depending upon the prior estrogen treatment (Caligaris et al., Endo. 88:810, 1971). Similar biphasic effects of estrogen on unit activity in the hypothalamus and preoptic area have not been clearly established. The present study was undertaken to investigate the effects of estrogen upon spontaneous hypothalamic and preoptic unit activity in estrogen-primed and non-primed ovariectomized rats. Extracellular potentials were recorded from paralyzed Sprague Dawley and Wistar Female rats with points of contact and incisions locally anesthetized with procaine.

In the medial preoptic area (MPOA) and anterior hypothalamic area (AHA) single unit activity was generally found to be depressed following a single estrogen administration (30 ug/kg estradiol benzoate in oil i.m.) compared to unit activity in the non-primed ovariectomized preparation. A substantial decrease in the number of units with response rates greater than one pulse per second was noted following a single estrogen administration. In the estrogen-primed rat, unit activity in the MPOA and AHA following a second estrogen administration was significantly elevated, with the majority of units recorded demonstrating firing rates greater than one pulse per second. These preliminary results suggest estrogen can have both depressive and facilitative effects upon hypothalamic and preoptic unit activity depending upon prior estrogen treatment.

1100 SEXUAL DIMORPHISM IN DENDRITIC PATTERN IN BASAL FOREBRAIN REGIONS OF INTACT ADULT HAMSTERS. W. T. Greenough, C. S. Carter, T. Ackerman*, R. Reeder*, J. Mateer*, T. Reeder*, E. Lyerla*, M. Bohnsak*, P. McCabe*, C. Suits*, and T. DeVoogd. Depts. Psychol. and Ethol., Ecol., and Evol. and Program in Neural and Behavioral Biology, Univ. Illinois, Champaign, IL 61820.

Prior work by our group and others indicates sexual dimorphism in connectivity patterns in rodent dorsomedial preoptic area (dmPOA). Our prior study (Greenough et al., Brain Res., 1977, 126: 63-72) indicated that dendrites of dmPOA neurons in male hamsters were more centrally concentrated than those in females. That study used animals gonadectomized in infancy or adulthood. We have now studied two-dimensional reconstructions of dendritic patterns of Golgi-Cox stained neurons from dmPOA and suprachiasmatic nucleus (SCN) in intact male and female hamsters. Dendritic distribution around each neuron was quantified in terms of the number of intersections between dendrites and an overlying grid of 20 micron equivalent squares. Dendritic distribution of groups of cells was reconstructed by adding grid intersection coordinates to cell body location coordinates (to 100 micron accuracy; ref. above). For dmPOA, the distribution paralleled prior work: males showed a central concentration while females showed a more widely distributed pattern of dendrites. This was true whether or not dendritic values were averaged relative to underlying cell body locations. Non-averaged distributions for males and females differed significantly at $p < .001$ ($\chi^2 = 1757.5$, df = 155). This suggests that our previously reported sex differences are not seriously affected by adult hormonal differences. In SCN, only dorsal and lateral neurons were analyzed due to sizeable sex differences in the number of neurons staining in ventromedial areas. The highest concentration of female dendrites was aligned along a ventromedial to dorsolateral axis, whereas male dendrites were, again, more centrally concentrated. This was emphasized by one-dimensional projection of the intersections to an oblique line orthogonal to this axis (slope = 0.5 mediolateral units per dorsoventral unit). Distributions along this axis differed at $p < .001$ ($\chi^2 = 37.4$; df = 7). In addition, analysis of the location around individual cell bodies where dendrites terminated revealed a sex by location effect within SCN, such that dendrites of male neurons tended to project toward the center of SCN, regardless of cell body position, whereas female dendrites were more evenly distributed or tended toward the above-noted oblique axis. Studies of the development of these differences and of possible dimorphism in anterior hypothalamus are in progress.

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1101 CHANGES IN POLYAMINE SYNTHESIS IN THE BRAIN AND THE LIVER OF THE RAT FOLLOWING ABLATION OF THE PITUITARY, ADRENAL OR THYROID GLANDS. Sami I. Harik. Dept. of Neurology, University of Miami School of Medicine, Miami, Fl. 33152.

Liver ornithine decarboxylase (ODC) activity and putrescine and spermidine levels in the liver and the brain were studied in hypophysectomized, adrenalectomized and thyroidectomized rats and in normal controls. Spermidine levels were decreased in the liver of all the endocrinopathic groups of rats while ODC activity and putrescine levels were not significantly altered. In the brain where ODC activity is not usually detectable, the levels of putrescine, which is the immediate product of the reaction catalyzed by ODC, was significantly increased in hypophysectomized and thyroidectomized rats. Brain spermidine was generally higher in all groups of endocrinopathic rats, but this increase did not reach statistical significance except in the thyroidectomized rats.

The chronic deficiency of hormones secreted by the pituitary, adrenal and thyroid glands does not seem to decrease the activity of ODC, which is the rate-limiting enzyme in polyamine synthesis.

Because of the intimate relationship between polyamine biosynthesis on one hand and nucleic acid and protein synthesis on the other, these results may help elucidate some aspects of growth and development under the effects of chronic hormone deprivation.

1102 BURSTING ACTIVITY IN RAT HYPOTHALAMIC NEUROSECRETORY CELLS IN VITRO IN RESPONSE TO OSMOTIC STIMULATION. Glenn I. Hatton, William A. Gregory, and William E. Armstrong. Dept. Psych., Mich. St. Univ., E. Lansing, MI 48824.

Extracellular potentials were recorded from rat hypothalamic cells in brain slices, using 2M NaCl-filled glass micropipettes. Rats were killed by decapitation, brains were rapidly removed, the hypothalamus was blocked with a razor blade and chopped into 500 μm thick slices on a tissue chopper. Usually 6 slices were placed in a temperature controlled chamber, supplied with a humidified 95% O_2/5% CO_2 mixture, and bathed in 2 ml of media (Yamamoto, Exp. Brain Res. 1972, 14, 423) of osmolality ≈ 300 mOsm/kg. An infusion-withdrawal pump was used to exchange the media bathing the tissue for media of either the same or higher osmolality. A total of 69 such preparations yielded recordings from 252 cells, of which 116 were paraventricular (PV) and 128 were nucleus circularis (NC) cells. The eight remaining units were recorded from the pituitary stalk, the nucleus of the diagonal band, the anterior hypothalamic area and the arcuate nucleus. Median basal firing rates for PV and NC cells were 5/sec and 7/sec, respectively. Exchanging the media without change in osmolality resulted in either no change or decreases in firing rates. Exchanges with media of 310 mOsm produced patterns of phasic activity similar to those seen in neurosecretory cells in vivo in response to osmotic stimulation. At this time, most of our data during media changes have been obtained from NC cells. Bursting activity (rapid, phasically occurring increases in firing rate) was seen in 6% of NC cells prior to osmotic stimulation, whereas 55% of cells recorded after stimulation were classified as bursters. Rates within bursts commonly reached 15/sec with some as high as 20/sec. Some cells in both nuclei were monitored before, during and after osmotic stimulation, and were observed to alter their firing patterns from random spiking to bursting activity. Such activity was not seen in any cells from other brain areas. These results suggest that bursting activity may be an intrinsic property of mammalian hypothalamic neurosecretory cells and that it can be evoked by osmotic stimulation.

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1103 NEURAL TRIGGERS FOR THE RELEASE OF VASOPRESSIN IN THE MONKEY. James N. Hayward and Kanok Pavasuthipaisit*. Dept. Neurology & Neurobiology Program, The University of North Carolina, Chapel Hill, N.C. 27514.

The behavioral state of the monkey is a major determinant for the release of vasopressin (VP) from the neurohypophysis. In the chronically prepared, chamber-isolated rhesus monkey we recorded EEG, EOG and body movement and obtained blood samples from an indwelling cardiac cannula for measurement of plasma VP by RIA (Endocrinology 98: 965, 1976). The sitting rhesus exhibits a circadian rhythm of VP with peaks at midnight during sleep and lows at noon during waking. With dehydration mean levels of VP rise in parallel with dehydration even as the day-night difference remains constant. A nocturnal, sleep-related, non-osmotic neural trigger may produce these nocturnal peaks of VP. During a 3-min simulated capture in the naive sitting rhesus, plasma VP rises rapidly to 10-20 fold above control values. Several factors, including the duration of simulated capture, conditioning of the animal and the intensity of capture, determine the magnitude of VP elevation. The neural pathways involved in this release of VP during simulated capture are unknown. In order to evaluate the possible role of the amygdala in the neural control of VP release we applied electrical stimuli to the temporal lobe, the hypothalamus and the pituitary gland. In the amygdala-stimulated monkeys, the plasma VP which rose rapidly to peak values (50-100 times basal) at the end of stimulation fell abruptly to control levels in 30 min. In monkeys stimulated electrically along the final neuroendocrine pathway (hypothalamus and pituitary gland) there was a temporal pattern of VP rise and fall identical to that seen following amygdala stimulation and simulated capture. Blood sampling timed precisely to the onset and end of these neural stimuli was an important factor in our ability to detect the rise and fall in plasma VP. We suggest that in the conscious monkey, the neural stimuli associated with sleep, simulated capture and electrical stimulation of the amygdala can trigger the release of VP. (Supported, in part, by NIH Grant NS-13411 and a Sloan Foundation Grant to the Neurobiology Program).

1104 RESISTANCE OF THE PITUITARY-ADRENAL SYSTEM OF THE MOUSE TO HABITUATION. Michael B. Hennessy* and Seymour Levine. Dept. Psychiatry & Behav. Sci., Stanford Sch. Med., Stanford, CA 94305.

The pituitary-adrenal system of the mouse is known to be sensitive to a variety of stimuli including exposure to a novel testing apparatus. Since responsiveness to the apparatus may mask responsiveness to stimuli presented within it, animals are often repeatedly exposed to the testing situation prior to testing in order to habituate responsiveness to the apparatus. Experiments in our laboratory suggest that these procedures often do not result in habituation.

In Experiment 1, blood samples were collected from adult male mice after they had been either: removed rapidly from their home cage (basal level control condition), placed in an apparatus once for 30 min (novelty condition), or had experienced one of three procedures involving prolonged exposures to the apparatus (habituation conditions). Assay of blood for plasma corticosterone showed that no habituation had occurred in any of the habituation conditions. One habituation condition produced sensitization, increasing rather than decreasing responsiveness. The elevated corticoid levels did not appear to be a result of novelty since animals in the habituation conditions had extensive time to become familiar with the apparatus.

Experiment 2 attempted to determine if the elevated corticoid levels could be accounted for in terms of the handling involved in placing animals into the apparatus. One vs. repeated exposures (of the sequence which produced sensitization in Experiment 1) was factorially combined with handling only vs. handling plus apparatus-exposure. Animals were found to not respond to handling alone, whether handling occurred once or repeatedly. That is, sensitization, and responsiveness in general, did not occur unless animals experienced apparatus-exposure.

Experiment 3 varied the familiarity of the apparatus to the home cage. Plasma corticosterone levels showed a clear discrimination of three apparatuses in terms of their familiarity. Thus, while response to novelty does not seem sufficient to explain the elevated corticoid levels, responsiveness does seem to depend upon the degree of discrepancy between home cage and apparatus.

These experiments demonstrate: the difficulty of habituating pituitary-adrenal responsiveness in the mouse; that repeated exposures to an apparatus can result in increased rather than decreased corticoid levels; that the elevated corticoid levels are not a response to handling; and that while novelty does not appear to account for the elevated corticoids, increasing the discrepancy between the home cage and the apparatus increases responsiveness to the apparatus.

1105 MODULATION OF PAIN SENSITIVITY IN THE RAT BY ADRENOCORTICAL HORMONES. John P. Heybach* and Joan Vernikos-Danellis*. (SPON: W. R. Mehler). NASA, Ames Research Center, Moffett Field, Ca. 94035.

Adrenocortical insufficiency in humans has been reported to lead to increases in sensitivity (ie. decreases in detection thresholds) to taste and to olfactory stimuli which were reversed via treatment with prednisolone (1). Similarly, an increased sensitivity to olfactory stimuli, which was reversed by corticosterone treatment, has been reported in the rat (2). The present study was conducted to determine whether adrenal hormones may also modulate cutaneous sensitivity to a painful thermal stimulus. Adrenalectomy and the circadian variation of adrenal function were the test conditions used to determine the effect of endogenous physiological changes in circulating adrenocortical hormone levels on pain sensitivity. Adrenalectomized adult male rats showed markedly decreased latencies in the display of a paw-lick, and subsequently a jump response, when placed on a grid at 55°C. Although sham adrenalectomized rats displayed some degree of adaptation or tolerance to this stimulus, as evidenced by increasing response latencies across days, adrenalectomized rats did not show a similar increase with repeated exposures. Adrenal demedullation had no effect on either paw-lick or jump latencies. The data suggest that adrenocortical hormones are involved in mediating pain sensitivity and the development of tolerance to pain. They further suggest that the rat is a useful model for studying the mechanisms of involvement of adrenocortical hormones in sensory processes.

1. Henkin, R. I. and Daly, R. L. (1968) J. Clin. Invest. 47, 1269.
2. Sakellaris, P. C. (1972) Physiol. Behav. 9, 495.

Note: J. P. H. is currently a National Academy of Sciences Research Associate.

1106 IMMUNOCYTOCHEMICAL EVIDENCE OF A STRUCTURAL SIMILARITY BETWEEN PINEAL ANTI-GONADOTROPHIC SUBSTANCE AND LUTEINIZING HORMONE RELEASING HORMONE (LHRH). G. E. Hoffman, K. M. Knigge*, and S. A. Joseph*. Dept. Anatomy, Sch. Med., Univ. Roch., Roch., N.Y. 14642.

The rat pineal gland in culture produces nanograms per day of substances with chemical characteristics (heat stability, extractability in methanol and acetic acid, proteolytic enzyme inactivation, and chromatographic mobility) suggestive of a small peptide similar to LHRH. Bioassay of this pineal substance by incubation with dispersed cultured rat anterior pituitary cells revealed no release of LH or inhibition of LHRH stimulated LH release. When melatonin was separated from the media, or its synthesis prevented by 100 µgram/ml p-chlorophenylalanine (PCPA), the pineal substance inhibited LHRH stimulated LH and FSH release. Immunocytochemical reaction of brain LHRH antigen-antibody reaction, using the unlabeled antibody peroxidase method of Sternberger, was inhibited when pineal media was present during incubation of antisera with tissue or when the antisera was pre-incubated with the pineal media. No inhibition of staining was seen when antisera was pre-incubated with melatonin or PCPA media incubated in the absence of pineals. Pineal substance was capable of displacing I^{125} LHRH from binding to anti-LHRH in the radioimmunoassay. The behavior of this pineal hormone with respect to LHRH antibodies and its chemical properties thus suggest this small peptide has as its primary structure, amino acid sequences which provide antigenic determinants similar to LHRH. (Supported by NIH fellowship HD 1 F22 00640).

1107 EFFECT OF CENTRAL CATECHOLAMINE DEPLETION ON CENTRALLY MEDIATED CARDIOVASCULAR RESPONSES. W.E. Hoffman*, J. Buggy, P.G. Schmid. Cardiovascular Division, Dept. of Internal Medicine, U. of Iowa, Iowa City, IA 52242.

Angiotensin II (A II), when delivered intraventricularly (IVT) produces an increase in blood pressure and release of antidiuretic hormone (ADH). In experiments reported here we have investigated the role of central catecholamines in these responses. Blood pressure was measured in chronic unanesthetized rats. ADH was determined by an on-line bioassay. After central 6-hydroxydopamine (6-OHDA) treatment IVT A II was less effective in producing a blood pressure increase of ADH release. Similar responses to IVT carbachol were also inhibited. Pressor responses to phenylephrine were not significantly changed after 6-OHDA treatment although ADH release was decreased. In further analysis, both a dopamine blocker, haloperidol, and phentolamine, an α-adrenergic blocker inhibited the pressor response but not ADH release to IVT A II. Both effects may be explained by α-adrenergic blockade. Dopamine IVT alone was ineffective in producing either ADH release or a blood pressure increase and phenylephrine produced both effects in a dose dependent manner. We conclude that noradrenergic mechanisms may be important as a common mediator of central sympathetic outflow. The ADH release produced by A II and carbachol may be by direct action on periventricular receptors which can be damaged non-specifically by 6-OHDA.

1108 THYROTROPIN RELEASING HORMONE - A PHYSIOLOGICAL β ENDORPHIN ANTAGONIST? John W. Holaday*, Choh Hao Li*, and Horace H. Loh* (SPON: Harry C. Holloway). Dept. Pharmacology, UCSF, San Francisco, CA 94143.

β Endorphin (BE) has been shown to be a potent hypothermic agent when injected intraventricularly (i.v.). Conversely, Thyrotropin Releasing Hormone (TRH) results in hyperthermia when administered i.v. We were interested in ascertaining whether or not TRH would antagonize the thermoregulatory as well as cataleptic, sialogogic, and antinociceptive effects of BE and to assess these effects in hypophysectomized rats.

Male Long-Evans rats, weighing 190 ± 10 (SD) grams, were hypophysectomized (hypox) or sham operated 10 days before testing. BE (30 µg in 20 µl saline) was injected i.v. through a guide cannula into the lateral ventricle. Pharmacological measures, accomplished in the 26.5°C room where housed, were continued until drug effects ceased.

This dose of BE had no significant effect on rectal temperatures of sham animals, whereas hypox rats experienced a 4°C decrease in temperature 3 h after injection - returning to normal at 5 h. Naloxone (4 mg/Kg) completely reversed this hypothermia when given 30 min after BE. TRH (20 µg in 20µl saline i.v.), injected 30 min after BE, also blocked the BE induced hypothermia in hypox rats, yet produced mild hypothermia in BE pretreated sham animals. Hypox rats, treated with TRH alone, become more hyperthermic than sham controls. Thus, hypox sensitizes to the thermoregulatory effects of both BE and TRH.

We have shown that BE (i.v.) initially results in wet-dog shakes followed by copious salivation, occasionally accompanied by a clonic seizure-like state in rats (manuscript in preparation). These behaviors were related to an altered thermoregulatory set-point. BE induced salivation in sham animals is decreased by TRH but potentiated by TRH in hypox rats. TRH alone causes wet-shakes in hypox rats to a greater extent than in sham controls. Conversely, BE alone causes more wet-shakes in sham animals than in hypox rats. These findings are consistent with the known BE hypothermic effects and the TRH hyperthermic effects.

Catalepsy induced by BE in both hypox and sham rats is antagonized by subsequent TRH injection. This is similar to previous reports of TRH antagonism of neuroleptic-induced catalepsy (Kruse, J. Pharmacol. (Paris) 6, 249, 1975). However, BE induced antinociception as measured by tail-flick is unaltered by TRH. Furthermore, TRH alone is without effect on tail-flick latencies.

Collectively, these studies support the hypothesis that a physiological interplay between TRH and endorphins may serve to modulate body temperature and other behaviors. Supported in part by Walter Reed Army Inst. Rsch., NIH, and NIDA grants.

1109 EFFECTS OF PRENATAL PROGESTERONE ON BEHAVIOR OF RATS. Elaine M. Hull, Jonathan Franz*, and Abigail Snyder*. Dept. Psychol., S.U.N.Y., Buffalo, N. Y. 14226.

Recent reports that administration of progesterone to women during pregnancy results in higher than average IQ among offspring or in increased "independence" measured on personality tests have raised a question concerning the potential mode of action of prenatally administered progesterone on the brain. At present no animal model of these effects has been demonstrated. Coyle, et al. (1977) have reported no statistically significant differences on numerous anatomical, physiological, and behavioral measures, though prenatal progesterone-treated rats did exhibit more rearing behavior and a greater amount of DNA in brain assays.

In the present experiment low (4mg), medium (8mg), and high (12mg) doses of progesterone were embedded in a Silastic pellet which was implanted subcutaneously in female rats on the sixth day of pregnancy. Control animals received plain Silastic pellets. 120 offspring of 12 females were tested on one-way active avoidance on Day 14 and on open field activity, Lashley III maze acquisition, one-way active avoidance, and passive avoidance beginning at 90 days of age. Animals born to medium-dose and control females performed best on the adult active avoidance task, compared to those born to low- and high-dose mothers. Significantly more medium-dose offspring than any other group obtained mean scores of 60 sec or faster on their last 5 Lashely III maze trials. While medium-dose animals obtained somewhat faster scores on the infant active avoidance task, the differences were not statistically significant. There were no Progesterone-related differences on open field activity, passive avoidance or weight at weaning. In summary low and high doses of prenatal progesterone impaired performance on two tasks, while the medium dose animals performed as well as controls on one task and better than all other groups on another.

1110 ACTIVITY OF ARYLAMIDASES IN THE RAT BRAIN INACTIVATING GONADOTROPIN RELEASING HORMONE AND THE EFFECT OF NEONATAL ANDROGENIZATION OF FEMALE RATS. I. A. Kamberi*, R. V. Wenn* and J. de Vellis (SPON: R. A. Gorski). UCLA, Sch. Med., Los Angeles, CA 90024.

Arylamidase activities towards specific substrates, the 4-nitroanilides of L-alanine, L-leucine, L-phenylalanine, L-tyrosine, L-cystine, L-glutamic acid, and L-glycine, have been investigated in different regions of the hypothalamus of normal male and female rats and neonatally androgenized female rats (1 mg testosterone propionate on day 3 after birth). In all hypothalamic regions examined (anterior, median eminence, posterior, lateral), it was found that the arylamidase activities with respect to these substrates decreased in the order listed above. The arylamidase activity towards L-alanine-4-nitroanilide and L-cystine-bis(4-nitroanilide) was elevated in all hypothalamic tissues as compared to the cerebral cortex. However, the relative distribution of the activity towards these two substrates varied in different regions with more pronounced activity in the anterior hypothalamus. The arylamidase activity was considerably higher in neonatally androgenized female than in normal diestrus female rats. There was no significant difference between activity in male rats and androgenized female rats. Hypothalamic gonadotropin releasing hormone (Gn-RH) was decreased in androgenized females as compared to normal diestrus females. Hypothalamic concentration of Gn-RH and serum levels of luteinizing hormone (LH) were not significantly different in neonatally androgenized female and normal male, but were much lower than in diestrus female rats. These results suggest that androgen treatment produces masculinization through activation of a mechanism responsible for Gn-RH inactivation in the hypothalamus. This in turn is reflected in changes in the gonadotropin secretion in neonatally androgenized female rats. (Supported by ERDA Contract EY-76-C-03-0012 and USPHS Grant HD-05615)

1111 ESTRADIOL-INDUCED ACTIVITY IN THE FEMALE RAT: EFFECTS OF LIMBIC AND DIENCEPHALIC LESIONS. James M. King. Dept. Psychol., University of Texas at Arlington, Arlington, TX 76019*

Female albino rats were given daily access to activity wheels for 1 hr during the dark portion of a reversed light-dark cycle. Following a 15 day baseline period, all subjects were ovariectomized and given sham lesions or bilateral lesions of one of the following sites: medial preoptic area (MP), anterior hypothalamic nucleus (AH), or corticomedial anygdala (CM). After surgery, subjects were given either daily subcutaneous injections of 3.0 micrograms of estradiol benzoate (EB) in sesame oil or the oil alone. After a 15 day recovery period, 15 additional days of activity data were collected. The EB enhancement of activity was sharply attenuated following MP lesions, and was entirely eliminated following AH lesions. CM lesions had no effect on EB-induced activity. A second study revealed that neither MP nor AH lesions interfered with the induction of activity by food deprivation, suggesting that the observed deficit is specific to EB-induced activity.

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1112 ECDYSONE TITERS IN NATURALLY AND BRAIN EXTRACT-INDUCED MOLTING MANDUCA SEXTA. Timothy G. Kingan* and R.W. Newburgh*. (SPON: Dorothy H. Paul). Dept of Biochem. & Biophys., Oregon State Univ., Corvallis, OR 97331.

The titer of the insect molting hormone, ecdysone, has been measured by radioimmunoassay (RIA) in the hemolymph of Manduca sexta larvae undergoing an L_4-L_5 molt. Evidence of hormone release is seen within three hours after "lights off" in larvae staged to initiate molting with prothoracicotropic hormone (PTTH) release on the day of selection. Ecdysone RIA activity increases six-fold within 18 hours of "lights off". Hemolymph ecdysone titers in similarly staged neck-ligated larvae were examined in response to injections of extracts of wandering stage larval brains. A significant increase ($P<.05$) in ecdysone titer occurs within one hour after injection when compared both to time = 0 and to control (2% NaCl injected) animals. The finding that the presumed primary endocrine response to PTTH release can be measured by ecdysone RIA may facilitate the characterization of PTTH. (Supported in part by NIH grant NS-09161-20.)

1113 EFFECTS OF GAMMA-BUTYROLACTONE AND OTHER ANESTHETICS ON GONADOTROPIN AND LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) LEVELS IN THE OVARIECTOMIZED ESTROGEN-PROGESTERONE PRIMED FEMALE RAT. John H. Kitchen* (SPON: K. M. Knigge). Dept. Anatomy, Univ. of Rochester Sch. Med., Rochester, N.Y. 14642

Gamma-hydroxybutyrate (GHB) is a naturally occurring product of gamma-aminobutyric acid (GABA) metabolism within the central nervous system. Clinically, GHB has been used to produce a type of anesthesia described as resembling 'natural' sleep or an epileptiform-like stupor. Gamma-butyrolactone (GBL) is converted by a blood lactonase to GHB, the active substance in inducing the unconscious state.

Ovariectomized, estradiol benzoate primed (EB, 10 μg s.c.) female rats were intraperitoneally injected, on the morning of progesterone administration (P, 1.5 mg s.c.) with GBL, urethane or pentobarbitol, using doses sufficient to produce surgical anesthesia. Animals were sacrificed by decapitation at various times after the injection of the anesthetics. Appropriate control animals were also sacrificed. Gonadotropin (sera, pituitaries) and LH-RH (brain punches) were determined by radioimmunoassay.

GBL did not block the afternoon rise in sera gonadotropin levels, in contrast to the results of urethane and barbituate anesthesia. LH-RH content, measured in the anterior preoptic and median eminence arcuate regions, did not significantly differ ($p < 0.05$) in any of the groups tested. Preliminary results suggest GBL does not alter blood pressure. The effects of GBL on central catecholamine content in discreet anatomically defined areas is currently being investigated using a radioenzymatic assay.

GBL, used alone or as an anesthetic adjuvant, may prove beneficial in experiments requiring a long-term anesthetic (electrophysiological recording, hypophyseal portal blood collection) which does not exert adverse or blocking actions on the reproductive neuroendocrine circuits.

1114 RHYTHM IN PINEAL MELATONIN EXCRETION IN HUMANS: DISRUPTION FOLLOWING CERVICAL SPINAL CORD LESIONS. *L.W. Kneisley[1,2], M.A. Moskowitz[2,3], H.J. Lynch[3], *H.R. Tyler[2], *A.B. Rossier[2]. From the Research and Spinal Cord Injury Services, West Roxbury Veterans Administration Hospital, Boston, Mass.[1], Section of Neurology, Peter Bent Brigham Hospital, Harvard Medical School[2], and Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Mass.[3].

In order to determine the extent to which disrupting the nervous innervation of the pineal organ alters the biosynthesis and secretion of melatonin in man, levels of this hormone were measured in the urines of subjects with traumatic spinal cord lesions. Urine samples were collected every four hours for twenty-four hours from 3 young male subjects with clinical evidence of complete cord transection above the C8 level. Melatonin excretion was determined for each interval by radioimmunoassay. Sleep was monitored by electroencephalography and serum samples were drawn from an indwelling catheter for additional neuroendocrine studies. The levels of melatonin in the urine during the waking state ranged from 0.3-1.5 ng/hr; those during sleep and darkness ranged from 0.3-1.5 ng/hr. In contrast to previously reported data from neurologically healthy adults, the pattern of excretion among patients with quadriplegia due to cervical cord lesions did not exhibit diurnal variations. One explanation for this failure to display a daily rhythm relates to the disruption of descending spinal sympathetic fibers that innervate the pineal organ via the intermediolateral horn of the spinal cord and superior cervical ganglia. Relative immobility and inactivity of these patients secondary to motor paralysis may also be a contributing factor. These findings suggest that in man as well as other animals, an intact spinal cord is necessary for generating a daily rhythm in melatonin biosynthesis.

1115 CONVERSION OF ANDROGENS TO ESTROGENS (AROMATIZATION) IN DISCRETE REGIONS OF THE RAT BRAIN: SEXUAL DIFFERENCES AND EFFECTS OF CASTRATION. Ronald M. Kobayashi and Ken C. Reed*. VA Hosp., San Diego, Dept. Neurosci. UCSD, La Jolla, CA 92097 and Dept. Biol. City of Hope, Duarte, CA 91010*.

Conversion of androgens to estrogens by aromatization of the A ring has been demonstrated in different brain regions. We report the localization of aromatase activity to discrete regions of the rat brain using a specific radioisotopic assay based on the release of ^3H_2O from [1β-^{3}H]-androstenedione. High sensitivity permitted measurement in tissue removed from individual animals by the microdissection technique of Palkovits.

Aromatase activity was distributed unevenly throughout the preoptic, hypothalamic and limbic regions. Highest activity in the male rat was measured in the n. preopticus medialis (npm), n. preopticus periventricularis (npp) and n. amygdaloideus medialis (nam). In the female, aromatization was highest in the nam. Activity in the median eminence, n. arcuatus and n. ventromedialis (nvm) was low to intermediate in both sexes. Discrete localization was striking, with activity in the npm 10-13x higher than in the adjacent n. preopticus lateralis and nam activity was 11-20x greater than in the adjacent n. amygdaloideus corticalis (nac).

Sexual differences were significant in 5 regions (npm, npp, n. preopticus suprachiasmatis (npsc), n. hypothal. ant. and nac). Activity in the male was 2-4x higher than in the female. Castration significantly reduced aromatase activity in the npm, npsc, nac and nvm, with decrease to levels resembling the intact female. No sexual difference in aromatase was present in the nam.

These results indicate that aromatase activity in the adult rat brain is discretely localized especially to the medial preoptic and medial amygdaloid regions, sexual differences are present and castration reduced activity in some regions. Aromatization in these loci may be relevant to sexual differentiation and regulation of sexual behavior. (Supported by VA Clinical Investigator Award and NIMH 26072 to RMK and USPHS-Australian National University Fellowships to KCR).

1116 GONADOTROPHIN SURGES INDUCED BY ADRENAL AND OVARIAN STEROIDS IN THE IMMATURE RAT. Ilze Kraulis*, K.B. Ruf, D. Lee* and F. Naftolin*. Dept. OB/GYN, Royal Victoria Hospital, McGill Univ., Montreal, P.Q. H3A 1A1, Canada.

Sexually immature female rats "primed" with estrogen release large amounts of LH and FSH in response to progesterone (Caligaris et al., J. Endocr. 55: 97, 1972; ibid. 58: 547, 1973); the resulting gonadotrophin surge resembles the one underlying spontaneous pubertal ovulation in this species. We have used this model to investigate the possible role of aromatizable androgens (known to be secreted by the adrenal) in estrogen-priming and of other adrenal steroids in the triggering of the preovulatory surge. Sprague-Dawley rats (23 d old) were injected with various androgens (0.6 mg or 6.0 mg/100 g b.w., s.c. for 3 days) and challenged with progesterone (1 mg) on day 26 (1200 h). Plasma was collected 5 h later and LH and FSH concentrations were measured by radioimmunoassay. Uterine weights, determined at the same time, served as cumulative index of prior exposure to estrogen. Rats primed with the higher dose of dehydroepiandrosterone or Δ^4-androstenedione exhibited peaks of similar magnitude (5 - 20x baseline) as animals primed with a single injection of 10 ug estradiol benzoate (EB) on day 23. The degree of uterine hypertrophy was also comparable in all groups. In the lower dose, the above androgens were ineffective, but massive gonadotrophin peaks were found after priming with 0.6 mg testosterone. 5α-Dihydrotestosterone and 3β,17β-androstanediol proved ineffective in either dose. A single injection of an antiserum raised against estradiol-17β in sheep abolished or greatly reduced the priming efficiency of EB and of the aromatizable androgens. In EB-primed rats, massive gonadotrophin surges could also be elicited by the administration of $ACTH^{1-24}$ (0.1 mg) or DOCA (1.5 mg). It is concluded that sexually immature rats are able to convert androgens in sufficient quantities to replace estrogen in sensitizing the hypothalamo-pituitary axis to the gonadotrophin-releasing effect of progesterone. Moreover, the data indicate that adrenal steroids other than progesterone might participate in triggering the pubertal preovulatory gonadotrophin surge in suitably primed animals. The adrenal is thus capable of contributing substantially to both components of the surge system.

1117 SUBCELLULAR LOCALIZATION OF ANTERIOR PITUITARY AND HYPOTHALAMIC 5α-DIHYDROPROGESTERONE 3α-HYDROXYSTEROID DEHYDROGENASE ACTIVITY. James E. Krause* and Harry J. Karavolas. Dept. of Physiol. Chem., Univ. of Wis., Madison, WI 53706.

Progesterone is metabolized by the anterior pituitary and hypothalamus to 5α-pregnane-3,20-dione (5α-dihydroprogesterone) and 3α-hydroxy-5α-pregnan-20-one. 5α-dihydroprogesterone has been proposed as a mediator of progesterone's neuroendocrine effects on gonadotropin regulation. The second enzyme in this metabolism of progesterone, the 3α-hydroxysteroid dehydrogense (3α-HSD), catalyzes the conversion of 5α-dihydroprogesterone to 3α-hydroxy-5α-pregnan-20-one. Thus, establishing the intracellular location of the enzyme involved in the metabolism of 5α-dihydroprogesterone may help to define the locus of 5α-dihydroprogesterone action.

The subcellular distributon of female rat anterior pituitary and hypothalamic 3α-HSD activity was investigated utilizing ^{3}H-5α-dihydroprogesterone as substrate and a reverse isotopic dilution assay system. Both NADPH and NADH stimulated 3α-HSD activity in whole homogenates, although stimulation by NADPH was greater. The NADPH and the NADH enzymatic activities could be separated and localized into distinct intracellular compartments using subcellular fractionation by differential centrifugation. Hypothalamic NADH dependent 3α-HSD activity was localized in a cell debris-membranes fraction derived from the 1000 x g pellet. 3α-HSD activity was absent in purified nuclei. Hypothalamic NADPH dependent 3α-HSD activity was present in the 105,000 x g supernatant. In contrast to the hypothalamic activity, anterior pituitary NADH dependent 3α-HSD activity was present in the 17,000 x g pellet and was localized further to a fraction containing tropic hormone secretory granules. As in the hypothalamus, anterior pituitary NADPH dependent 3α-HSD activity was present in the 105,000 x g supernatant. (Supported by grants from NIH and the Ford Foundation.)

1118 AXONAL PROJECTIONS FROM THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN THE RAT. Monica S. Krieger*, Lily C. A. Conrad* and Donald W. Pfaff. The Rockefeller University, New York, New York 10021.

Previous work from this laboratory has demonstrated that estrogen-concentrating cells exist in the ventromedial nucleus of the hypothalamus (VMH) in a number of vertebrate species. Other researchers have implicated the VMH in the control of gonadotropin secretion and reproductive behavior. A way to help elucidate the roles of estrogen-concentrating cells and other VMH cells in controlling gonadotropin secretion and reproductive behavior is to describe VMH efferent connections. We have done this using tritiated amino acid autoradiography.

Injections of 10 nl of a concentrated solution of ^{3}H-leucine (200 μCi/μl) were made into the VMH using stereotaxic procedures. Forty-eight hours after injection into VMH, rats were perfused with formalin. The brains were removed, embedded in paraffin, sectioned and mounted on slides. These slides were dipped in Kodak NTB-3 nuclear emulsion, exposed for 30 or 45 days and then developed. Projections from VMH neurons were charted systematically, using light and dark field microscopy to analyze the autoradiograms.

Fibers ascending from VMH passed through the preoptic area and the diagonal bands of Broca. Diffuse silver grains were seen in the bed nucleus of the stria terminalis and the lateral septal nucleus. Fibers going dorsally ran toward the periventricular thalamus, or entered the stria terminalis and projected to the amygdala. Some descending fibers coursed medially through the premammillary region and swept dorsally in the posterior hypothalamus through the periventricular system into the mesencephalic central gray. Other descending fibers coursed laterally. These included labelled fibers in a strong, bilateral projection in the ventral supraoptic commissure, some fibers of which entered the amygdala. Terminations were found in the medial, cortical, lateral nuclei and in the area surrounding the central nucleus of the amygdala. Other supraoptic commissure fibers cut through the cerebral peduncle and more caudally they swept through the thalamic reticular nucleus and over the dorsal thalamus toward central gray. There was also a lateral descending projection through the zona incerta which continued into the lateral mesencephalic reticular formation. Although in most cases it is difficult to interpret intrahypothalamic connections there appeared to be a projection from VMH to the median eminence.

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1119 THE FACTITIOUS ROLE OF SEROTONIN ANTAGONISTS ON PROLACTIN SECRETION. S. W. J. Lamberts* and R. M. MacLeod*. (SPON: J. I. Kitay). Dept. of Medicine, Univ. of Virginia, Sch. Med., Charlottesville, VA 22901

Normal prolactin (PRL) secretion is under the tonic inhibition of dopamine (DA). We previously showed a direct inhibitory effect of DA on PRL secretion at the pituitary level. In contrast, the serotoninergic system is thought to be stimulatory toward PRL secretion because the administration of 5-HTP stimulates the secretion of the hormone. In this study, we analyzed the action of certain serotonin antagnoists on PRL synthesis and release in vitro by incubating rat pituitary glands with ^{3}H-leucine. The amount of newly synthesized and radioimmunoassayable PRL in the incubation medium and pituitary was determined. Serotonin (0.1-10 μM) in vitro did not effect PRL synthesis or release in the pituitary of male and female rats. The inhibitory effect of dopamine (500 nM) on PRL release in vitro was unchanged in the presence of serotonin (6 μM). A serotonin antagonist, methysergide (3 nM), had no in vitro effect on PRL secretion or synthesis, but it blocked the dopamine-mediated inhibition of PRL release. Another serotonin antagonist, cyproheptadine (60-600 nM), had a significant inhibitory effect on the release of ^{3}H-PRL (35 and 81%, resp.), while the release of radioimmunoassayable PRL was inhibited to a lesser extent (24-55%); the sum total of RIA-PRL in the medium and pituitary together was unchanged by cyproheptadine suggesting that the primary effect of cyproheptadine is on PRL secretion. The effect of cyproheptadine was dose-dependent and the IC_{50} was 0.24 μM (0.4 μg). The addition of 25 ng TRH to control pituitary gland incubations stimulated PRL secretion 40%. Although TRH produced a similar degree of stimulation in the presence of .58 μM cyproheptadine, the peptide did not restore hormone secretion to control levels. In contrast to the effects seen with methysergide the co-incubation of cyproheptadine and DA had an additive inhibitory effect on PRL secretion. We have previously demonstrated that the inhibitory effect of DA and its agonists was blocked by various neuroleptic agents. Contrary to this, the inhibitory effect produced by cyproheptadine was not blocked by haloperidol (50 nM), pimozide (50 nM) and perphenazine (50 nM). It is concluded that the stimulating effect of serotonin on PRL secretion is mediated through some hypothalamic mechanism and not directly on the pituitary. Methysergide has properties of a dopamine agonist and antagonist, depending upon the test system. Cyproheptadine is an inhibitor of PRL secretion, but this effect is not mediated through the dopaminergic system.

1120 CORTICOSTERONE MODIFICATION OF THE SYNTHESIS OF SPECIFIC PROTEINS IN THE CENTRAL NERVOUS SYSTEM. Kevin S. Lee*, Anne M. Etgen*, and G. S. Lynch. (SPON: E. Greene). Dept. Psychobiology, University of California, Irvine, CA 92717.

Corticosterone, the principal glucocorticoid secreted by the rat adrenal cortex, is selectively bound and retained by certain limbic structures, especially the hippocampus. The majority of this hippocampal binding is localized within neuronal nuclei, as demonstrated by autoradiography and subcellular fractionation techniques. Specific, high-affinity cytoplasmic and nuclear receptors for corticosterone have also been isolated from the hippocampus. However, intracellular actions of this steroid hormone on its central nervous system (CNS) targets have not been identified. Since glucocorticoids act as intracellular modulators of gene expression in many peripheral tissues by regulating protein synthesis, the present investigation sought to determine if corticosterone modifies protein synthesis in its primary CNS target, the hippocampus.

Transverse slices of hippocampus prepared from adult male Sprague Dawley rats were maintained at 32-34°C in a balanced salt medium. Protein synthesis was measured by the incorporation of ^{3}H-leucine into protein during a 1-hour incubation with or without 10^{-9}M corticosterone added to the medium. The total protein synthesis rate in control and treated slices was determined by calculating the amount of labeled leucine incorporated into TCA-precipitable protein. The rate of synthesis of specific proteins was evaluated by fractionating proteins from several subcellular fractions on SDS-polyacrylamide gels. In some experiments, slices of cerebral cortex, a tissue which does not bind corticosterone, were coincubated with the hippocampal slices in order to assess the specificity of the hormone's action.

Corticosterone did not alter total protein synthesis in either the hippocampus or the cortex. However, the 1-hour hormone treatment consistently (18/20 experiments) and significantly ($t=4.21$; $df=19$; $p<.001$) increased the synthesis of at least one soluble cytoplasmic protein in the hippocampus by an average of 12 percent over controls. This protein has an apparent molecular weight of approximately 53-55,000 Daltons. Hormone treatment did not affect the synthesis of any cortical proteins.

Corticosterone thus altered the synthesis of specific protein(s) in a central target tissue without effecting any change in total protein synthesis. These results provide the first evidence that steroids can regulate specific protein synthesis in the CNS as well as in peripheral target organs. These observations are discussed in light of the current model of steroid hormone interaction with the genome. The possible mediation of glucocorticoid effects on brain electrical activity and behavior through hormonal regulation of specific protein synthesis is also suggested.

1121 DIFFERING NEUROCHEMICAL AND HORMONAL RESPONSES TO SPECIFIC STRESSORS. R. H. Lenox, J. L. Meyerhoff, G. J. Kant, G. R. Sessions, L. L. Pennington*, and E. H. Mougey*, Depts. of Medical Neurosciences and Microwave Research, Walter Reed Army Inst. Rsch. Washington, DC 20012.

It has been observed that the hormonal response to stress varies depending upon the nature of the specific stressful stimulus (Mason, Psychiat. Res. 8 (1971) 323). In the rat various stressors have been found to increase plasma corticosterone and prolactin and to decrease growth hormone levels. Cold stress in rats has been shown to cause increases in cyclic GMP (cGMP) in several brain regions (Mao, Molec. Pharm. 10 (1974) 736). We have compared the response of several neurochemical and hormonal systems to immobilization or cold exposure.

Male albino rats, WRC strain, weighing between 250-300 gm, were maintained in a light-cycled chamber and habituated to both handling and placement into a plexiglass cylinder twice a day for 1 week. On the day of sacrifice, animals were placed into one of three groups. The first group was placed in the plexiglass holder and immediately sacrificed by a high power microwave irradiation system, modified in our laboratory (Lenox, IEEE Microwave Theory Tech. 24 (1976) 58). The second group was immobilized for 5 minutes in the plexiglass holder, before irradiation. The third group was exposed to cold (4°C, with fur wetted) for 5 minutes and then irradiated. Immediately following exposure to the microwave irradiation, animals were decapitated and blood was collected in heparinized containers.

Levels of cGMP in specific brain regions were significantly elevated only in animals exposed to cold stress. The cGMP increase in cerebellum was approximately 3 fold with significant increases also noted in 9 other regions. Cyclic AMP (cAMP) levels throughout the 16 regions of brain examined showed no significant response to either of the stressors. In the pituitary, the level of cGMP was elevated in animals exposed to cold stress, while the cAMP level tended to increase only in the immobilized group. In the regions noted, the cGMP response to cold exposure was not affected by bilateral lesions of the locus coeruleus. Plasma prolactin levels were elevated over 10 fold in the immobilized group, while only rising 2 fold in the cold stress group. Levels of corticosterone were significantly increased while growth hormone levels tended to decrease in both the immobilized and cold stress groups.

It would appear from our data that regional brain cGMP and plasma prolactin respond differentially to 2 stressors while plasma corticosterone and growth hormone respond less specifically. The specifics of the stressful stimuli may be crucial in determining the nature of the neurochemical and hormonal response to stress in the rat.

1122 RESPONSE PATTERNS ELICITED BY α-MSH IN CENTRAL DOPAMINE NEURONS:ROLE OF ACTIVE SITES,DOSE AND HORMONAL STATE. Walter Lichtensteiger and Florianne Monnet*,Inst. Pharmacol.,Univ.Zürich,CH-8006 Zürich, Switzerland.

In ovariectomized, estrogen-progesterone-pretreated rats, acute systemic administration of α-melanotropin (α-MSH) has been found to affect the functional state of the tubero-infundibular and nigrostriatal dopamine (DA)neuron systems(Lichtensteiger and Lienhart,Nature 266,635, 1977).In both neuron groups,the peptide elicited the same response,an increase in neuronal fluorescence intensity as assessed by microfluorimetry, which indicates neuronal activation.In preliminary experiments,ACTH 4-10 was able to induce a similar reaction. In male rats,the response patterns are more complex and differ in part between the two DA systems. In the nigral group, a dose of α-MSH effective in females remained without effect or tended to reduce neuronal fluorescence(or activity),whereas a lower dose(ineffective in females)provoked a response of the type seen in females. The tuberal DA neurons responded to the whole dose range studied (2-100 μg/kg) by an increase in intensity.Differences between the two DA systems were further noted when their response(30 min) to comparable doses of peptide fragments containing the central and C-terminal active site of the α-MSH molecule, i.e.,ACTH 4-10 and MSH 11-13 (acetyl-Lys Pro Val NH_2),was investigated.In males, ACTH 4-10 exerted some effect on the nigral DA neurons but remained largely ineffective on the tuberal system.In contrast, the tuberal but not the nigral DA neurons exhibited a rise in fluorescence intensity after MSH 11-13.

The results indicate that the hormonal state of the animal strongly influences the response of central DA systems to peptides of the α-MSH type. Moreover, the two active sites of the α-MSH molecule appear to be of unequal importance for the two DA systems. Together with our earlier observations on differences in the neuronal circuitry underlying the peptide effect, this finding suggests that the physiological significance of the reaction differs between the two DA systems. The assessment of a possible involvement of the pituitary in these peptide effects is complicated by marked effects of hypophysectomy itself especially on the tuberal DA neurons. Supported by SNSF grant 3.669-0.75.

1123 HALF-CYLINDER CUTS ANTERO-LATERAL TO THE VENTROMEDIAL NUCLEUS REDUCE SEXUAL RECEPTIVITY IN THE FEMALE HAMSTER.# C.W. Malsbury, D.Strull* and J.Daood*. Western Psychiatric Inst.& Clinic, Dept. Psychiatry, Univ. Pittsburgh Sch. Med.,Pittsburgh, Pa. 15261

Previous data had shown that lesions in the region of the ventromedial nucleus of the hypothalamus (VMN) can disrupt the lordosis response in female golden hamsters (Malsbury, Kow & Pfaff, Physiol. & Behav.,in press). In the present study a Halász-type knife was used to help determine the connections of the medial basal hypothalamus that are critical for this response.

Ovariectomized, estradiol benzoate plus progesterone treated females were given weekly tests for lordosis before and after hypothalamic cuts. The vertical and lateral dimensions of the cuts were approximately 1.5 and 1.2 mm respectively. It was found that complete MBH isolations produced by a 360° rotation of the knife blade can eliminate lordosis. However, when VMN tissue was left outside the cut lateral, or anterior, lordosis could still be elicited. One-half cylinder cuts (180° knife rotation) were made either antero-lateral or postero-lateral to the VMN. Some of the antero-lateral cuts disrupted lordosis as completely as full-cylinder cuts, while postero-lateral cuts had little or no effect. The 360°, and 180° antero-lateral cuts passed through, or posterior to the suprachiasmatic nuclei on the midline, while postero-lateral cuts passed through the mammillary bodies or interpeduncular region. The behavioral data are summarized below. We conclude that neural pathways critical for lordosis pass in or out of the VMN region in an antero-lateral direction.

Lordosis Performance Following Medial Hypothalamic Knife Cuts†

			180° Cuts			
Group (N)	Unop. Control (10)	360° Cut (6)	Antero-Lateral (9)	Postero-Lateral (7)	A-L Sham (5)	P-L Sham (3)
Manual	94(±11)	40*(±20)	50(±16)	93(±7.1)	99(±1.3)	88(±7.2)
Male	105(±7.0)	27*(±21)	23*(±13)	81(±10)	86(±5.8)	77(±5.8)

†The mean (±S.E.M.) post-lesion lordosis performance in each group was calculated as: (mean post-lesion lordosis duration/mean pre-lesion lordosis duration) x 100. Total lordosis duration was measured in response to 5 min. of continuous manual stimulation and 5 min. of exposure to a sexually active male.

* Significant difference from unoperated control group (Mann-Whitney U test, $p < .05$, two-tailed.)

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1124 SUBFORNICAL ORGAN: SITE OF PRESSOR AND DRINKING EFFECTS OF ANGIOTENSIN II. Michael L. Mangiapane* and John B. Simpson. Depts. of Psychology and Physiology, Univ. Washington, Seattle, WA 98195

The area postrema, a circumventricular organ, has been implicated as a central site of angiotensin II (A II) pressor action in some species. However, Hoffman and Phillips (Brain Research, 110, 1976) reported that lateral and rostral third ventricular A II injections produced maximal pressor responses in the rat with concurrent water ingestion; the fourth ventricle was insensitive. We now report that the subfornical organ (SFO), a site known to contain receptors for the drinking effect of intracranial A II (Simpson and Routtenberg, Science, 181, 1973), also mediates a pressor response to intracranial A II. In unanesthetized, freely moving rats prepared with intracranial cannulae and chronic aortic catheters, the pressor and drinking effects of A II injection were compared at three loci: SFO, adjacent ventral fornical commissure (VFC), and dorsal third ventricle (III V). At 10 ng, 1 ng, and 0.1 ng of A II (0.2 ul injection volume), SFO injections were clearly the most effective, producing pressor responses of 24, 19, and 9 mm Hg with concurrent drinking of 7.6, 5.8, and 3.8 ml H_2O. Both effects were blocked by the A II antagonist, Saralasin Acetate (Norwich). Saline vehicle injections had neither dipsogenic nor pressor effects. Adjacent to SFO, VFC and III V injections at these doses of A II produced responses less than or equal to half of the SFO drinking and pressor responses. Further, mean SFO pressor latencies (from injection until onset of blood pressure increase) ranged from 4-13 sec; significantly longer latencies were recorded at the other two loci. Injections into SFO invariably produced a significant pressor response which was not secondary to the act of drinking. Smaller A II doses which, in some cases, did not elicit drinking did provoke a pressor response; further, the onset of the pressor effect always preceded the onset of water ingestion. It is important to note that SFO cannulae did not rupture ventricular ependyma at any point, and that ventricular A II injection was less effective than SFO injection of A II. Ventricular access of A II thus seems to be unnecessary for drinking or pressor effects. These results suggest that the neurons of one circumventricular organ, the SFO, may mediate both behavioral and physiological responses to A II, and establish that pressor activity emanates from this structure.

1125 INHIBITION OF LORDOSIS BY INTRACRANIAL IMPLANTS OF PROGESTERONE IN THE FEMALE RAT. B. L. Marrone*, J. F. Rodriguez-Sierra* and H. H. Feder* (SPON: W.B. Quay) Institute of Animal Behavior, Rutgers-The State University, Newark, NJ 07102.

Progesterone implants in the medial-basal hypothalamus (MBH) have been reported to facilitate lordosis in ovariectomized, estrogen-primed rats and guinea pigs. In the present set of experiments we examined the lordosis facilitatory and inhibitory effects of progesterone implants in the MBH of rats.

Ovariectomized rats were implanted with unilateral, double barreled cannulae aimed at regions in and adjacent to the MBH. One week after surgery implants of progesterone or cholesterol were inserted a) 1 hr prior to a priming dose of estradiol benzoate (EB, 5 ug/Kg) followed 44 hr later by 0.5 mg progesterone (concurrent inhibition) or b) 44 hr after a priming dose of EB (facilitation) followed by 0.5 mg of progesterone at 68 hr (sequential inhibition). All animals were allowed 10 mounts by a male rat at 44 hr and 48 hr after EB. Animals tested for sequential inhibition also received tests at 68 hr and 72 hr after EB. Lordosis quotients (lordoses/10 mounts x 100) were computed for each test.

No facilitatory effects of progesterone implants were observed in any site tested. However, both concurrent and sequential inhibition of lordosis were observed when progesterone was implanted in the MBH. Progesterone implants lateral, anterior, or dorsal to this region or cholesterol implants were ineffective.

Results show that the MBH is an area responsive to inhibition, but not facilitation of lordosis by progesterone. In addition, the similarity of sites responsive to concurrent and sequential inhibition suggest that these two inhibitory effects of progesterone on sexual behavior are achieved via the same mechanism.
(Supported by HD-04467)

1126 CORRELATIVE FLUORESCENCE-IMMUNOCYTOCHEMICAL LOCALIZATION OF MONOAMINES AND NEUROPHYSIN, VASOPRESSIN AND GONADOTROPIN-RELEASING HORMONE IN THE RAT AND MONKEY HYPOTHALAMUS. Thomas H. McNeill and John R. Sladek, Jr. Dept. Anat., University of Rochester School of Medicine, Rochester, N.Y. 14642.

A technique has been developed for the correlative localization of monoamines and neurohormones on sections of freeze-dried tissue in the mammalian hypothalamus. Adult male and female rats and monkeys were used for this experiment. Brains were excised after decapitation and the diencephalon was dissected and frozen in freon-22 prechilled in liquid nitrogen. Tissues were freeze-dried in a Sladek-Kontes freeze drier, exposed to paraformaldehyde vapors and embedded in paraffin. Tissues were sectioned at 10μm and mounted on glass slides for fluorescence examination, with adjacent sections mounted for immunocytochemistry. The unlabeled antibody peroxidase anti-peroxidase technique was used for the localization of neurophysin (NP), vasopressin (VP), and gonadotropin-releasing hormone (Gn-RH). The distribution of the immunoreactive precipitates for these neurohormones and the monoamine fluorophors was similar to what has been described previously in the rat and monkey hypothalamus using these two techniques separately. Catecholamine varicosities were seen, for example, within the preoptic area, the supraoptic (SON) and paraventricular (PVN) nuclei, the median eminence and elsewhere. Fluorescent perikarya of the A-12 and A-14 dopamine cell groups also were observed. Despite the absence of fixation prior to freeze-drying, NP, VP, and Gn-RH were localized in perikarya and axonal varicosities within the hypothalamus. Catecholamine varicosities were found adjacent to neuronal perikarya of the SON and PVN. This apparent axosomatic juxtaposition suggests a possible interaction between catecholamine terminals and NP-containing perikarya. NP and VP terminals were seen principally in the zona interna of the median eminence, however, beaded fibers were found in the zona externa next to the portal capillaries. Gn-RH terminals were seen in linear profiles adjacent to fluorescent tanycytes. This combined fluorescence-immunocytochemical technique provides, for the first time, a correlative method for the study of the interaction between neurotransmitters and neuropeptides on adjacent sections, which will allow for the further elucidation of the role of these substances in the regulation of the peripheral endocrine system.

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1127 EFFECT OF D- AND L-AMPHETAMINE ON RAT SERUM PROLACTIN LEVELS. H.Y. Meltzer, M. Simonovic, R. Fessler, and V. Fang. Dept. of Psychiatry and Med., U. Chicago Schl. Med., Chicago, IL. 60637.

Secretion of prolactin from the rat pituitary is under tonic inhibition by dopamine (DA) released into the pituitary portal circulation. Thus, drugs which increase dopaminergic activity at the pituitary: e.g. L-DOPA, apomorphine, decrease rat plasma prolactin levels. Amphetamine (AMP) increases the release of DA, blocks reuptake of DA, and prevents its catabolism by inhibiting monoamine oxidase activity. Thus, AMP should decrease plasma PRL levels. However, Lu and Meites (Proc. Soc. Exp. Biol. Med., 137, 480, 1971) reported that D-AMP, 5.0 mg/kg i.p. markedly increased serum prolactin levels in female rats, while decreasing pituitary prolactin levels. However, Horowski and Gröf [Acta Endocr. (Kbh) Suppl., 199, 203, 1975] reported that D-AMP, 10 mg/kg subcutaneously, diminished the increase in serum prolactin produced by reserpine but not reserpine plus alpha-methylparatyrosine (AMPT), an inhibitor of DA synthesis. D-AMP itself had no effect on serum prolactin levels in male rats.

We have studied the effect of D- and L-AMP on male rat plasma prolactin levels to determine the relative potency of each isomer in antagonizing the increase in prolactin produced by RES and AMPT. This would help determine the relative effect of each isomer on dopaminergic mechanisms. D- or L-AMP in doses of 0.5, 1.0, 2.5, 5 and 25 mg/kg was given 3 1/2 hr after RES 5 mg/kg i.p. The increase in PRL was partially antagonized by D-AMP even at the lowest dose and was completely antagonized by all doses greater than 2.5 mg/kg i.p. L-AMP 25 mg/kg completely antagonized the increase in PRL produced by RES. Our results suggest that the effect of D-AMP on increasing DA levels at the pituitary is 5-10 times greater than that of L-AMP.

Neither D- or L-AMP, at doses up to 5 mg/kg i.p. had any effect on the increase in rat plasma prolactin produced by AMPT-methyl-ester 100 mg/kg i.p. given 30 min prior to AMP. On the other hand, apomorphine, 5 mg/kg i.p., and LSD, 0.1 mg/kg i.p., both DA agonists, readily inhibited the AMPT-induced increase in rat plasma prolactin. These results are consistent with other studies indicating D-AMP and L-AMP are indirect DA agonists while apomorphine and LSD are direct DA agonists.

Chronic treatment with D-AMP for 10 days with doses up to 10 mg/kg i.p. did not affect the ability of D-AMP to antagonize the increase in prolactin produced by RES 5 mg/kg i.p. This is in accord with previous results indicating that tolerance does not develop to the effects of D-AMP on DA mechanisms.

1128 EFFECTS OF AN ENDORPHIN ANALOG ON THE HABITUATION OF AN INNATE FEAR RESPONSE IN GOLDFISH. Gary Michell*, Richard D. Olson, Abba J. Kastin, Gayle A. Olson*, Dolores Montalbano*, and David H. Coy*. Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70122

After an initial adaptation period in a tall, narrow tank, goldfish typically swim up and down at a fairly constant rate. If a loud buzzer is sounded during the ascent, however, the fish will either become immobile and remain near or actively swim to the bottom of the tank. Upon repeated experience with the buzzer, this innate response habituates and the rate of ascent and descent gradually approaches baseline.

Using this paradigm, fish were administered a 5 µl (8µg/kg) IP or ICV injection of the endorphin analog (D-Ala-2-β-endorphin) or the diluent control. Half of each endorphin group was tested without the buzzer as a control for immobilization. Results indicated that while the latencies of ascent increased reliably for all endorphin groups, they were not significantly different from each other. Thus it appeared that the primary effect of endorphin was immobilization. For the fish exposed to the buzzer versus those not exposed to the buzzer, latencies after endorphin were virtually identical when collapsed over trials. In addition to the typical trials effect indicative of habituation, a reliable peptide-by-trials interaction was obtained due to the very long initial latencies and the short terminal latencies. A significant injection-by-trials interaction appeared to be accounted for by an intense effect of the peptide in ICV groups at the start of testing followed by a rapid dissipation over trials. Activity scores obtained during a 15-minute adaptation period showed all endorphin groups to be reliably less active than controls. Furthermore, IP groups were less active than ICV groups. All activity differences disappeared during the subsequent testing period which followed immediately. Finally, total testing time did not differ between groups. These data were consistent with the basic finding that endorphin had an intense immobolizing action, and that IP injections required additional time to manifest the effect.

This work was supported in part by the VA and NIH:NS07664.

1129 PINEAL AND GONADAL RESPONSES TO LIGHT IN THE DEVELOPING RAT. Robert Y. Moore, Nicholas A. Vick and Richard L. Rapport. Dept. of Neurosci., Univ. Calif., San Diego, La Jolla, CA 92093.

The pineal gland in the rat exhibits an active metabolism of indolamines which is regulated by central circadian rhythm generating mechanisms, synchronized by the diurnal cycle of light and dark. The product of this metabolism, melatonin, is dependent upon the activity of the pineal enzymes, serotonin N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT). The NAT rhythm develops early, appearing by 6 days of age and reaching adult values by 21 days of age. In contrast to NAT, pineal HIOMT activity is high in animals maintained in constant darkness (CD) and low in animals maintained in constant light (CL; Wurtman et al, 1963) but shows no significant diurnal fluctuation (Klein, 1974). Pineal HIOMT activity reaches adult levels late in comparison to NAT (Klein and Lines, 1969).

The present study was carried out to determine if a relationship exists between the development of the pineal HIOMT response to light and gonadal function in the rat. Two groups of pups were used; one consisted of animals raised from birth in CD and the other in CL. Each group contained a subgroup of sham operated pups and a group of pinealectomized (PX) pups. The operations were performed on day 6. Both CD and CL control animals show very low levels of pineal HIOMT activity until day 12 when an abrupt increase occurs. HIOMT activity then gradually increases in both CD and CL animals until day 32. At that point, CL animals exhibit pineal HIOMT of about 150 pmoles melatonin formed/gland/hr and this remains constant thereafter. CD animals show increasing pineal HIOMT between days 32 and 36 to approximately twice that of the CL animals. Female rats raised in CD show vaginal opening on day 43.8 ± .4 whereas CL female rats show vaginal opening on day 37.1 ± .1. CD-PX animals show vaginal opening on day 37.6 ± 1.2 co-pared to CL-PX, 36.2 ± .3. Similarly, ovarian weights were nearly identical in all groups, about 35 mg/100 mg body weight except for the CD sham operated group, 23.2 ± 1.2 mg/100 mg body weight. Male rats exhibit similar differences in seminal vesicle weight.

These data suggest that the pineal HIOMT response to light may play a significant role in the control of puberty in the rat. (Supported by USPHS Grant NS-12267)

1130 AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-ESTRADIOL, ^{3}H-TESTOSTERONE AND ^{3}H-DIHYDROTESTOSTERONE IN THE BRAIN OF THE LIZARD, ANOLIS CAROLINENSIS. Joan I. Morrell, David Crews[1]*, Arleen Ballin* and Donald W. Pfaff. The Rockefeller University, New York, New York; [1] Harvard University, Boston, Massachusetts.

The neuroanatomical pattern of sex steroid concentrating cells in the brain of the lizard, Anolis carolinensis, was demonstrated with the autoradiographic method. Studies in a wide variety of vertebrates (Morrell et al., '75) have shown that brain areas of greatest cellular concentration of sex steroids are frequently involved in neuroendocrine mechanisms of reproduction or sex behavior.

Healthy female and male (weight range 2.3 to 5.1 gms) Anolis were gonadectomized, and one month later the animals were injected with 4µCi/gm body weight of either ^{3}H-estradiol (9 animals), ^{3}H-testosterone (5 animals) or ^{3}H-dihydrotestosterone (4 animals) (S. A. 80-100 Ci/mMole). Two hours later the animals were sacrificed, the brains removed and frozen. Autoradiograms and control sections were prepared using the methods previously published by this laboratory. After five or nine months exposure the autoradiograms were developed, and scanned systematically.

After ^{3}H-estradiol administration many labelled cells were found in specific telencephalic and diencephalic structures. The largest number of labelled cells are seen in the medial and lateral preoptic areas; in the anterior, ventromedial and posterior areas of the hypothalamus; and in the amygdala. The anterior pituitary contains a large number of extremely heavily labelled cells. The medial and lateral septum and a cellular group just dorsal to the lateral forebrain bundle, at the level of the preoptic area contain large populations of labelled cells. Many labelled cells are found in the lateral telencephalic cortex, and in the ventral striatum between the lateral forebrain bundle and the amygdala. Some labelled cells are found in the dorsal and medial telencephalic cortex. The mesencephalon contains a number of labelled cells in the torus semicircularis, some in the tectum and in the tegmentum. The rhombencephalon contains labelled cells in several distinct groups, although the number of cells is small compared to the number in the forebrain.

After ^{3}H-testosterone or ^{3}H-dihydrotestosterone injection, locations of labelled cells are in general similar to those following ^{3}H-estradiol injection, but in most areas the number and/or intensity of labelled cells was clearly less. For all three hormones, no differences in concentration pattern were seen when males and females were compared. The nature of diencephalic and telencephalic sex hormone binding in Anolis reinforces the notion of a vertebrate-wide pattern of sex hormone uptake by neurons (see Morrell, et al., '75; Brain-Endocrine Interaction II, Karger).

1131 NEONATAL SUPRACHIASMATIC NUCLEUS LESIONS IN THE RAT: EFFECTS ON THE DEVELOPMENT OF CIRCADIAN RHYTHMICITY AND THE RETINOHYPOTHALAMIC PROJECTION. Sarah S. Mosko and Robert Y. Moore. Dept. Neurosciences, UCSD, La Jolla, CA 92093.

Bilateral ablation of the suprachiasmatic nucleus (SCN), the terminal nucleus of the retinohypothalamic (RH) projection, in the adult rat results in the loss of a number of circadian rhythms. In the present study, the SCN was destroyed in the neonatal rat to determine whether the developing RH projection would exhibit plasticity and innervate another hypothalamic nucleus and whether the developing CNS can establish circadian rhythms in the absence of the SCN. The SCN was ablated bilaterally in newborn rats prior to the arrival of the RH projection from the eye. When adult, females subjected to neonatal SCN lesions fail to show normal estrous cycles and exhibit long periods of constant vaginal cornification. Both females and males show a loss of circadian rhythmicity in locomotor activity and drinking. Autoradiographic analysis of the developing RH fibers reveals a high degree of specificity of the fibers for their normal target tissue. In the absence of the SCN, no evidence is found for an aberrant projection to other areas, e.g., nearby hypothalamic sites. However, partial SCN ablation often results in hyperinnervation of the surviving SCN fragment(s). In correspondence with the normal topography of innervation, retinal fibers retain a remarkable specificity for the caudal portion of the nucleus and some degree of preference for its ventral and lateral aspects. These findings emphasize the developmental significance of the SCN in circadian rhythm generation and demonstrate, first, a great developmental specificity in the RH projection and, second, that in contrast to other components of the visual system, neither sparing of function nor developmental plasticity occurs after early SCN lesions. (Supported by USPHS Grant NS-12267)

1132 CHEMOSENSITIVITY OF HYPOPHYSIOTROPIC NEURONS TO THE MICROELECTROPHORESIS OF BIOGENIC AMINES. R.L. Moss, M.J. Kelly* and C.A. Dudley*. Dept. Physiol., Univ. of Tx. Hlth. Sci. Cntr., Dallas, TX 75235.

Determination of the catecholaminergic and cholinergic sensitivity of neurons projecting from the medial-preoptic (MPO) and arcuate-ventromedial (ARC-VM) nuclei to the median eminence was undertaken due to the implied involvement of such neurons and putative transmitters in the regulation of anterior pituitary function. The action of norepinephrine (NE), dopamine (DA), serotonin (5-HT), acetylcholine (ACH) and glutamate (Glut) when applied in the immediate vicinity and in minute quantities via microelectrophoresis to single MPO and ARC-VM neurons was studied in the urethane anesthetized, ovariectomized female rat. Multibarrelled glass micropipettes were utilized for extracellular recording and for microelectrophoresis of the putative transmitters.

Four specific neuron types were identified by electrical stimulation of the median eminence, namely, antidromically identified (AI) MPO neurons, uninvaded MPO neurons, AI, ARC-VM neurons and uninvaded ARC-VM neurons. The major findings based on responsive neurons, i.e., those neurons which reacted to the drug application by an increase or decrease in spontaneous activity are reported. Examination of the data revealed that NE had different effects on neurons in the two areas studied. The majority of AI, ARC-VM (72%) and uninvaded ARC-VM (69%) neurons exhibited a marked increase in firing rate to the application of NE. However, responsive AI, MPO neurons were inhibited by NE application [(-)24%; (+)7%] and uninvaded MPO neurons had a similar percentage of excitatory (32%) and inhibitory (27%) responses. The influence of DA was not as clearly defined, although there was a slight trend towards excitation in uninvaded MPO neurons [(+)30%; (-)22%] and inhibition in AI, MPO neurons [(-)25%; (+)16%].

Results based on the testing with 5-HT showed it to have an inhibitory action on uninvaded MPO neurons [(-)43%; (+)13%]. Furthermore, ACH and Glut were shown to have an excitatory action on the majority of responsive neurons tested. The remaining neurons tested with either NE, DA or 5-HT displayed a similar number of excitatory and inhibitory responses. The findings obtained provide positive support for a catecholaminergic and cholinergic regulation of neurons projecting towards the median eminence, which are presumably involved in pituitary function. (Supported by NIH Grant #NS10434)

1133 EFFECT OF LIGHTING ON MATURATION OF NEURAL ELEMENTS CONTROLLING OVULATION IN RATS. Osamu Nakamoto and Nobuyoshi Hagino. Dept. of Anatomy, U.T.H.S.C.S.A., San Antonio, Texas 78284.

In rats (Sprague-Dawley) born in a light-dark schedule (LD), the onset of puberty is 39.4 days of age and regular estrous cycles and spontaneous ovulation are observed. Plasma estrogen is elevated on proestrus prior to ovulation. When 22 day-old rats are transferred to continuous illumination (CI), precocious puberty occurs at 33.7 days and anovulation and persistent estrus occurs as adults. However, when these rats mate under CI, ovulation is induced and pregnancy is possible. Rats from these litters born under CI show normal onset of puberty at 39.9 days and have regular estrous cycles with ovulation even though maintained under CI. These rats exhibit a regular pattern of plasma estrogen similar to that shown by rats maintained under LD. It seems likely that LD is a predominant factor in regulation of the estrous cycle and ovulation when rats are subjected to LD during gestation and through puberty. However, when rats are subjected to CI during these periods, light is no longer a regulatory factor in regard to ovulation and estrous cycles.

Further investigation was made to examine the biorhythm of sleep and wakefulness in rats born and reared in CI. Sleep and waking patterns were assessed by EEG recordings from chronically implanted electrodes in the frontal cortex and dorsal hippocampus, and EMG recordings from neck electromyographic electrodes, together with observation of animal behavior. Recordings proceeded for four consecutive days with one daily interruption to obtain vaginal smears. Rats born and reared in LD exhibit circadian rhythm of EEG slow wave sleep (SWS), alertness (A), and paradoxical sleep (PS). However, when these rats are transferred to CI, circadian rhythm of SWS, A, and PS is annihilated and, indeed, all rhythms disappear. In contrast, rats born and reared under CI demonstrate rhythmatic SWS, A, and PS, but these patterns approach a reversed circadian rhythm.

This evidence indicates that in rats born and reared in LD, the neural elements of the light receptive area effectively modulate the structures controlling either pituitary-ovarian function or circadian rhythm of SWS, A, and PS. Therefore, when the light receptive area becomes refractory by exposure to CI, biorhythm is annihilated.

It may be considered that in rats born and reared in CI, the light receptive area becomes ineffective and other activating systems take over regulation of the estrous cycle, ovulation, and biorhythm of sleep and wakefulness. Furthermore, the activating systems involved in certain control of gonadotrophin secretion, as well as biorhythm of sleep and wakefulness, may be masked by more active participation of the light receptive area in rats born and reared in LD. (Supported by NIH HD 10071).

1134 REDUCED LEVELS OF IMMUNOREACTIVE LHRH IN GENETICALLY OBESE MICE (ob/ob). Charles B. Nemeroff, Garth Bissette*, and John S. Kizer*. Biol. Sci. Res. Ctr., Depts. Pharmacology and Medicine, Univ. North Carolina, Sch. Med., Chapel Hill, NC 27514

The recessively inherited obese-hyperglycemic syndrome in mice is characterized by marked obesity, insulin-resistance, and reproductive dysfunction. Previous studies (Swerdloff et al. Endocrinology 98, 1359, 1976) indicated a disruption in the hypothalamic-pituitary axis as the cause of the sterility and hypogonadism. Our results suggest that ob/ob mice, unlike normal controls (+/+) or heterozygotes (ob/+) have significantly lower levels of immunoreactive LHRH in the hypothalamus. C57 Bl/6J ob/ob, ob/+ and normal controls were purchased from Jackson Laboratories and maintained in an air conditioned, controlled lighting (14 h light, 10 h dark) animal facility and fed laboratory chow and water ad libitum. The animals were sacrificed by decapitation and the hypothalami from representative groups (n=5/group) were rapidly removed, wet weight recorded and assayed for LHRH, TRH, SRIF, DA or NE. The releasing hormones were assayed by radioimmunoassay (RIA) as previously described (Kizer et al., Endocrinology 98, 685, 1976); the catecholamines were assayed by radioenzymatic methods (Coyle and Henry, J. Neurochem. 21, 61, 1973). The results are shown in the table below. Data is expressed as ng/mg wet weight ± SEM.

	Controls	N	ob/ob	N	p
NE	0.79 ± 0.1	5	0.85 ± 0.1	5	ns
DA	0.51 ± 0.1	5	0.48 ± 0.1	5	ns
TRH	0.27 ± 0.04	10	0.25 ± 0.03	10	ns
LHRH	0.07 ± 0.01	10	0.04 ± 0.01	10	<0.01
SRIF	0.24 ± 0.08	5	0.23 ± 0.07	5	ns

In contrast to previous studies (Lordin et al., Brain Res. 96, 390, 1975), we found no statistically significant differences in hypothalamic catecholamine levels between ob/ob and control mice. Immunoreactive levels of TRH and SRIF were also unchanged in ob/ob mice. However ob/ob mice contained almost 50% less hypothalamic LHRH than normal mice. Lean littermate controls (ob/+) did not differ from normal controls (+/+) in any of the parameters measured. These results suggest that diminished LHRH levels in the hypothalamus of the ob/ob mouse may underly the reproductive dysfunction observed in these animals. (Supported by NINCDS NS-05722, NICHD HD-03110 and NIMH MH-28899).

1135 INHIBITION OF RENIN RELEASE BY α-ADRENERGIC STIMULATION. Peter L. Nolan* and Ian A. Reid* (SPON: W.F. Ganong) Dept. of Physiology, U.C.S.F., San Francisco, CA 94143

The antihypertensive drug clonidine suppresses renin secretion by acting within the central nervous system to decrease renal sympathetic nerve activity (I.A. Reid et al., J. Pharmacol. exp. Therap. 192, 713, 1975). It has also been proposed that clonidine inhibits renin secretion by stimulating intrarenal α-adrenergic receptors (W.A. Pettinger et al., Circ. Res. 38, 338, 1976). In the present investigation, the effect on renin secretion of oxymetazoline, a potent α-adrenergic receptor agonist which does not cross the blood brain barrier, was studied. Experiments were performed in pentobarbital-anesthetized dogs in which changes in renal perfusion pressure were prevented by adjusting a suprarenal aortic clamp.

In 13 dogs, oxymetazoline (1 μg/kg i.v.) decreased plasma renin activity (PRA) from 23.7±6.5 to 15.4±5.1, 15.1±5.6 and 15.0±4.6 ng/ml/3h after 15, 30 and 60 min respectively ($P < 0.02$). Mean arterial pressure (MAP) increased transiently but returned to the control value within 15 min. Heart rate (HR) decreased from 131±8 to 119±8 beats/min ($P < 0.02$). In 6 dogs pretreated with the α-adrenergic receptor antagonist phentolamine (0.5 mg/kg i.v. at -45 and -15 min) and then given oxymetazoline (1 μg/kg), PRA decreased transiently from 36.8±9.2 to 29.4±6.0 ng/ml/3h at 15 min but had increased to 34.6±6.5 and 32.2±5.7 ng/ml/3h by 30 and 60 min respectively. MAP and HR did not change significantly.

In a second series of experiments, the effects of clonidine were studied. In 6 dogs, clonidine (30 μg/kg i.v.) decreased PRA from 22.1±7.8 to 13.5±4.8, 8.4±3.4 and 6.9±2.8 ng/ml/3h after 15, 30 and 60 min respectively ($P < 0.05$). MAP increased from 124±8 to 133±7 mm Hg after 15 min but then decreased to 104±4 mm Hg after 60 min ($P < 0.01$). HR decreased from 131±10 to 79±5 beats/min ($P < 0.01$). In 5 dogs pretreated with phentolamine (0.5 mg/kg i.v. at -45 and -15 min), clonidine (30 μg/kg) lowered PRA from 40.8±6.6 to 18.4±3.7, 12.4±2.3 and 8.3±1.6 ng/ml/3h after 15, 30 and 60 min respectively ($P < 0.01$). Phentolamine pretreatment did not antagonize the hypotension or bradycardia produced by clonidine.

These data indicate that stimulation of peripheral α-adrenergic receptors with oxymetazoline inhibits renin secretion. On the other hand the renin-lowering action of clonidine was not reduced by phentolamine and it is therefore unlikely that stimulation of peripheral α-adrenergic receptors contributes significantly to this response.

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1136 RADIOIMMUNOASSAY FOR β-ENDORPHIN. Norio Ogawa*, Alberto E. Panerai*, Viktor Havlicek and Henry G. Friesen*. Dept. Physiol., Univ. of Manitoba, Winnipeg, Canada, R3E OW3.

β-endorphin (β-E), corresponding to aminoacid residues 61-91 of β-LPH, binds to the opiate receptor but the physiological significance of this peptide remains to be defined. A sensitive and specific radioimmunoassay (RIA) for β-E has been developed. Synthetic camel β-E (Peninsula Lab.) was conjugated with bovine serum albumin using glutaraldehyde. This complex was emulsified with Freund's complete adjuvant and injected into rabbits to generate antibodies. β-E was iodinated with ^{125}I using lactoperoxidase and H_2O_2 and purified on a carboxymethyl cellulose column using ammonium acetate buffers with a gradient from 0.002 to 0.6 M. Three or four radioactive peaks were eluted, the last peak exhibiting the greatest binding to antiserum. Antisera to β-E bound 55% of ^{125}I-β-E at a final dilution of 1:60,000 by the dextran-coated charcoal method. In the RIA, bound and free ^{125}I-β-E were separated by the double-antibody method. Sensitivity was usually 100 pg/tube. The cross-reactivity of this antiserum is <0.02% with Met^5-enkephalin, <0.01% with Met^5-enkephalin-amide (supplied by Dr. Niall), <0.02% with Leu^5-enkephalin, <0.01% with α-endorphin (supplied by Dr. Guillemin), <0.2% with β-LPH (supplied by Dr. Chrétien), 30% with human β-E and 0% with Naloxone, on a weight basis. Rat anterior plus intermediate and posterior lobes of the pituitary gland were incubated in Krebs Ringer bicarbonate buffer and the β-E concentrations were determined by RIA. The highest concentrations (20 fold greater) of immunoreactive β-E were found in the incubation medium from the posterior pituitary lobe. Immunoreactive β-E was also detected in rat brain using several different extraction methods. The absolute values differed markedly (5-10 fold) depending on the extraction procedure used. Acid extracts of rat pituitary and brain exhibited dose-response curves indistinguishable from that of synthetic β-E. (Supported by MRC, USPHS, and Non-Medical Use of Drugs Directorate, Canada.)

1137 EPILEPTIC DRUGS EFFECT ON ADRENAL CORTEX MATURATION IN THE RAT. Tony D. Okonmah*, Karam F. A. Soliman and Charles A. Walker. School of Pharmacy, Florida A&M University, Tallahassee, Florida 32307.

The effect of CNS stimulation was studied in relation to adrenal maturation. In this investigation, two different weanlings (day 22) Sprague Dawley female rats were placed on 14:10 light-dark cycle at a temperature of 23±1°C. At age 23 and 29 days, pentylenetetrazol (10.00mg/kg) was injected for three days in two groups, while the other groups were left as control. Blood samples were obtained for cortiscosterone determination every 4 hours along the 24 hour period. Result from both age groups indicate that pentylenetetrazol suppressed plasma corticosterone level. The peak level for the corticosterone release occurred during the dark phase in the CNS treated group. While pentylenetetrazol did abolish the corticosterone variation in the 23 day old rats, this was not true in the 29 day old rats. Corticosterone daily changes were correlated to the circadian variation in the ovaries and uteri weights. Peaks for both ovary and uterus occurred at the light phase in the control and the treated animals. In general, ovarian and uterine hypertrophy was observed in the pentylenetetrazol treated animals. The results from this work indicated that CNS stimulation can affect the adrenal corticosteroid diural rhythm and the maturation of adrenal-pituitary axis. Research supported by "NIH" grant, number RR8111.

1138 THE EFFECT OF CORTICOMEDIAL AMYGDALA LESIONS AND STEROID TREATMENT ON SERUM PROLACTIN LEVELS IN MALE AND FEMALE RATS. J.A. Peters* and R.R. Gala* (SPON: R.A. Barraco), Dept. Physiol., Wayne State Univ. Sch. Med., Detroit, MI 48201.

Pituitary prolactin secretion is known to be under inhibitory hypothalamic control, but the neuroendocrine regulation of this hormone by extrahypothalamic brain regions is poorly understood. The effects of bilateral radiofrequency lesions of the corticomedial amygdala (CMN) on serum prolactin (PR) levels were studied in adult male and female Sprague-Dawley rats, 250-350 g, housed under controlled lighting (14 hrs L; 10 hrs D) conditions. In one study adult males were lesioned 1 week after castration and injected either with 1.0 mg of polyestradiol phosphate (PEP), or no steroid 2 weeks later. Two weeks after PEP, castrated males (L_O), castrated PEP treated males (L_e), and similarly treated non-lesioned controls (C_O and C_e) were decapitated at 1100 hrs and 1700 hrs and serum PR levels measured by RIA. PEP significantly elevated PR in castrated males at both time periods. The positive feedback effect of estrogen on PR was significantly greater in L_e than C_e rats decapitated at 1100 hrs. A lesion in the absence of estrogen had no effect on basal PR levels in castrated males. In a second series of experiments, females were lesioned or sham lesioned 1 week after ovariectomy (OVX) and injected 2 weeks later with either 1.0 mg PEP or no steroid. Two weeks later the animals were bled either by decapitation or by aortic catheter at 1100, 1700, and 0400 hrs. PEP treatment elevated PR at all time periods when compared to OVX females, and produced a significant PR elevation at 1700 hrs. CMN lesions had no significant effect on basal or surge PR levels in OVX or OVX-PEP treated females. In another experiment, females were lesioned or sham lesioned 1 week after OVX and 3 weeks later injected with 1.0 mg testosterone propionate (TP) daily for 1 week prior to decapitation at 1100 and 1700 hrs. There was no effect of CMN lesion at either time period. TP induced a significant afternoon PR surge similar to that produced by PEP in OVX females. The 1100 hr PR levels, however, were significantly lower than those of OVX-PEP females and castrated PEP males, and similar to those of castrated males. Finally, neonatally androgenized (NA) females given 1.25 mg TP on day 3 postnatally were investigated as adults. Preliminary data in our lab indicates that bilateral CMN lesions lower the elevated serum PR levels of NA females to the levels seen in males. Our results suggest a role for the CMN amygdala in regulating the estrogen-induced elevation in PR levels in the male and NA female rat. The testosterone induced PR elevation does not appear to involve the CMN. (Supported in part by NSF Research Grant No. 74-17332, and by a WSUSM predoctoral fellowship to J.A. Peters.)

1139 CHANGES IN HYPOTHALAMIC LUTEINIZING HORMONE-RELEASING HORMONE (LH-RH) IN THE MALE GOLDEN HAMSTER IN RESPONSE TO SHORTENED PHOTOPERIODS. Gary E. Pickard. Neuroscience Program, Wis. Reg. Primate Res. Ctr. and Dept. Neurophys., Univ. Wis., Madison, Wis. 53706

Day length and testicular steroids interact to regulate the hypothalamo-adenohypophyseal neuroendocrine system involved in gonadotropin synthesis and secretion in the hamster. Shortened photoperiods (<12.5 hr/day) have been shown to be inhibitory to the reproductive system of the golden hamster (Mesocricetus auratus). This is reflected by the depressed serum gonadotropin levels accompanied by testicular regression. However, the site(s) and mechanism(s) by which non-stimulatory photoperiodic conditions inhibit the hypothalamo-adenohypophyseal neuroendocrine system are largely unknown.

Hypothalamic levels of LH-RH of adult male hamsters housed for 13 weeks either in long day (LD) (14:10) or short day (SD) (6:18) photoperiodic conditions were assessed by both radioimmunoassay (RIA) and immunocytochemical techniques. Specific antiserum to LH-RH was provided by S. Sorrentino, Jr. and the peroxidase-antiperoxidase (PAP) complex was supplied by L.A. Sternberger. Serum LH levels were monitered throughout the experimental period using a rat LH RIA system supplied by NIAMD.

Animals were sacrificed by decapitation and either the arcuate-median eminence (AR-ME) region was dissected out and homogenized in 90% methanol for RIA or the brains were fixed by immersion in Bouin's fixative for subsequent immunocytochemical examination.

After 13 weeks exposure to shortened photoperiods, serum LH and testes weight/100g body weight of SD animals were significantly reduced compared to LD controls. However, the AR-ME tissue of the SD males contained significantly higher concentrations of LH-RH assayable material compared to LD controls. In addition, LD castrates demonstrated decreased AR-ME concentrations of LH-RH compared to LD intact animals, but this decrease in hypothalamic LH-RH was not seen in SD castrates. Immunocytochemical examination of the LH-RH fiber system supported the RIA findings.

Male hamsters housed under non-stimulatory photoperiodic conditions demonstrated depressed serum LH values and testicular regression. The increased hypothalamic levels of LH-RH found in these animals suggest that one possible effect of shortened photoperiods on the neuroendocrine system may be to inhibit the hypothalamic release of LH-RH

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1140 SOMATIC, BEHAVIORAL, AND REPRODUCTIVE DISTURBANCES IN MICE FOLLOWING EARLY ADMINISTRATION OF SODIUM L-ASPARTATE.
William J. Pizzi, Josephine M. Tabor* and June E. Barnhart*. Neuropsychology Lab, Northeastern Ill. Univ., Chicago, IL 60625.

Neonatal administration of the dicarboxylic amino acids glutamate and aspartate has been shown to produce both retinal damage and CNS lesions. This report presents data demonstrating that neonatal administration of aspartate can produce adult mice that are obese, have reduced body length, are hypoactive, show reproductive dysfunction and have reduced endocrine organ weights.

96 neonatal mice were given subcutaneous doses of sodium L-aspartate (ASP) or bacteriostatic saline from days 2-11 after birth according to a dose schedule which starts at 2.2 mg/g b.w. and increases to 4.2 mg/g b.w. by the last day of injection. Animals were placed on ad lib food and water until they reached 150 days of age, at which time both male and female ASP-treated animals weighed more than controls (males, C=16, vs. E=26, $p < 0.001$); females, C=25, vs. E=29, $p < 0.001$).

Female reproductive function was assessed by creating mating environments composed of 2 control females, 2 ASP-treated females and 1 control male. Male reproductive function was tested by pairing 1 ASP-treated male with 1 control female. All matings started when the animals reached 150 days of age, and ended at 180 days of age. ASP-treated females failed to become pregnant as often as control females (C=16 of 16 vs. E=2 of 14, $p < 0.005$). ASP-treated males also failed to impregnate control females as readily as did control males (C=8 of 8 vs. E=3 of 11, $p < 0.005$). ASP-treated females showed delayed vaginal canalization indicating a delay in sexual maturation.

Male animals were tested on 2 measures of locomotor and exploratory activity starting between 190 and 195 days of age. ASP-treated males showed significantly decreased activity in both the open field test ($p < 0.025$) and the Boissier Poke Test ($p < 0.01$).

Upon autopsy ASP-treated males had significantly shorter body lengths along with significantly lower weights of pituitary, thyroid and testes. ASP-treated females showed the same pattern with smaller and lighter ovaries. Neither group showed differences in adrenal gland weights when compared with controls. All ASP-treated groups had significantly heavier body weights at the time of autopsy, and showed large accumulations of adipose tissue throughout the body.

These results demonstrate that the neurotoxic properties of aspartate produce the same sequalae of deficits seen in glutamate-treated animals; namely, obesity, decreased body lengths, hypoactivity, reproductive deficits and endocrine dysfunction.

1141 ACUTE ELEVATION OF SERUM LUTEINIZING HORMONE INDUCED BY LOW SYSTEMIC DOSES OF POWERFUL NEUROEXCITATORY ANALOGS OF GLUTAMATE.
M.T. Price, J.W. Olney and T.J. Cicero. Washington University School of Medicine, St. Louis MO 63110.

Glutamate (GLU), a putative excitatory transmitter, destroys neurons in the arcuate nucleus of the hypothalamus (AH) when administered subcutaneously (sc) to infant or adult rats, a brain damaging dose being < 0.5 g/kg in infancy or > 2 g/kg in adulthood. Olney et al. recently reported (Br. Res. 112, 240, 1976) that sc administration of a non-brain damaging dose of GLU (1 g/kg) to adult male rats results in an acute elevation of luteinizing hormone (LH). If, as we postulate, this effect is mediated by the excitatory action of GLU upon AH neurons, it should be possible to reproduce it by sc administration of excitatory analogs of GLU, especially those known to be more powerful excitants than GLU. To test this hypothesis, we administered DL-homocysteate (DLH), N-methyl-DL-aspartate (NMA) and kainate (KA) [listed in order of increasing excitatory potencies] to 25 day-old male Holtzman rats in sc doses from 1 to 100 mg/kg. Nacl-treated control rats were included in each set of experiments. A total of 312 animals were studied, all being sacrificed either for LH serum determination by radioimmunoassay at 7 1/2 min after injection or for histopathological examination of the brain at 3 hours.

Each experimental compound significantly ($p < .001$) increased serum LH levels when compared to controls. The lowest effective doses defined as those doses which produced statistically significant increases in serum LH levels, were 5 mg/kg (KA), 16 mg/kg (NMA) and 50 mg/kg (DLH). At double these doses, NMA and DLH did not damage the 25 day-old rat brain but KA sometimes induced extensive spreading lesions; therefore, NMA and DLH may be useful neuroendocrine probes but KA is probably too toxic.

These findings, together with evidence from microelectrophoretic and ultrastructural studies, suggest that GLU and its analogs interact with excitatory receptors on the dendritic or somal surfaces of AH neurons to stimulate firing of these neurons. Since at least some AH neurons are thought to be dopaminergic, stimulation of these neurons by GLU-mimetic excitants could involve the release of dopamine (DA) from their axon terminals and a dopaminergic mechanism might mediate hypothalamic secretion of luteinizing hormone releasing hormone (LHRH) into the portal vascular system to effect pituitary release of LH. It should be of interest, therefore, to determine whether the LH elevating effects of excitatory amino acids can be suppressed by DA receptor blocking agents. Supported by U.S. Public Health grants NS-09156, DA-00259 and RSD Awards MH-38894 (JWO) and MA-70180 (TJC).

1142 EFFECTS OF INTRACEREBROVENTRICULAR INJECTION OF DOG AND ARTIFICIAL RENIN SUBSTRATES ON DRINKING, BLOOD PRESSURE, VASOPRESSIN AND ACTH SECRETION IN DOGS.
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Recent evicence indicates that the enzyme with renin activity in brain is not active at extracellular fluid pH. However, intracerebroventricular (IVT) injection of synthetic tetradecapeptide renin substrate (TDP) has been reported to cause drinking in rats. This has been interpreted as evidence for brain renin activity in vivo. In the present experiments, the effects of IVT injections of TDP and natural dog renin substrate on drinking, blood pressure and the secretion of vasopressin and ACTH were compared.

Injection of TDP into the third ventricle of 7 dogs in doses of 100, 250, 500 and 1,000 ng initiated drinking of 70±24, 253±78, 448±71 and 472±143 ml H_2O respectively in a 30 min period. The latency of the effect was 1-5 min, similar to angiotensin II (AII). The drinking following 500 ng TDP was reduced to 63±22 ml ($P < 0.001$) by IVT injection of 2 µg of the AII antagonist, saralasin, and to 130±83 ml ($P < 0.001$) by IVT injection of 12 µg of the converting enzyme inhibitor, SQ 20881. Administration of the renin inhibitor pepstatin (10 µg IVT), however, did not reduce the drinking to 500 ng TDP (488±152 ml). These results indicate that the dipsogenic action of TDP is mediated via the formation of AII by brain converting enzyme.

Injection of 250, 500 and 1,000 ng of renin substrate prepared from dog CSF did not cause drinking, suggesting that addition of natural substrate to the CSF does not result in AII generation.

In a second series of experiments in 7 pentobarbital anesthetized dogs, the effect of 500 ng TDP and 500 ng natural substrate on CSF AII blood pressure and plasma vasopressin and ACTH concentrations were compared. Hormone levels were measured by radioimmunoassay. Central administration of dog substrate had no effect on arterial blood pressure, plasma vasopressin or ACTH levels. Furthermore, it was not associated with the appearance of AII in CSF. Administration of TDP, however, increased CSF AII concentration to more than 8,000 pg/ml. Blood pressure increased by 18 mm Hg and there were highly significant increases in plasma vasopressin and ACTH concentrations.

These results fail to demonstrate renin activity in dog brain in vivo and indicate that the central effects of TDP are due to formation of AII by converting enzyme.

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1143 ORGANIZATION OF THE SUPRACHIASMATIC NUCLEUS IN THE ALBINO RAT.
Joseph N. Riley and Robert Y. Moore. Dept. Neurosciences, Univ. Calif., San Diego, La Jolla, Calif. 92093.

A direct projection from the retina to the suprachiasmatic nuclei (SCN) of the hypothalamus has been demonstrated in a number of species. Destruction of the SCN abolishes a number of circadian rhythms, suggesting that the SCN may function as a CNS generator of circadian rhythms. It seemed worthwhile, therefore, to examine the morphological organization of the SCN and the retinohypothalamic projection in detail. The organization of the SCN of Sprague-Dawley rats was examined in Nissl and Golgi-Kopsch material. The retinal projection to the hypothalamus was examined in autoradiographic material prepared after intraocular injections of 3H-proline or 3H-adenosine.

Each nucleus contains approximately 10,000 neurons. The cellular packing density is higher in the ventromedial region. The approximate maximum dimensions of each nucleus are 1.6 mm in length and 0.6 mm in diameter.

Most neurons have 2 or 3 primary dendrites that usually branch only once. Neurons appear to differ in the number of observed dendritic protrusions. The orientation of dendrites differs in various regions: ventral neurons usually have dendrites oriented parallel to the optic chiasm in both saggital and transverse planes; dendrites of dorsally located neurons usually are oriented dorsoventrally. Some evidence has been obtained in Golgi material that neurons located immediately lateral to the nuclei project to the SCN.

The autoradiographic material from intraocular injections of 3H-proline confirms and extends previous autoradiographic studies of the retinal projection to the SCN. The retinal projection is bilateral but heavier contralaterally. In addition to previously described projections to ventral regions, projections to the lateral regions of the SCN were observed. There appears to be no retinal projection to the first 400 µm of the nucleus. The projection is first seen ventrally, then ventrolaterally. The projection extends to the caudal portion of the SCN where numerous silver grains are present in a relatively cell free area surrounding the caudal pole of the nucleus. The lateral projection is denser and appears to extend further caudally on the side contralateral to the injection. The results from a preliminary analysis of 3H-adenosine material are consistent with the observations made on 3H-proline material, suggesting that the cells of the nucleus receiving the innervation are those in the ventrolateral part of the caudal three-fourths of the nucleus.
(Supported by USPHS Grants NS-12267 and NS-07086)

1144 MIDBRAIN CENTRAL GREY: AN EXTRAHYPOTHALAMIC SITE FOR LRH POTENTIATION OF LORDOSIS BEHAVIOR IN FEMALE RATS. Peter Riskind* and Robert L. Moss. Dept. Physiol., Univ. of Tx. Hlth. Sci. Cntr., Dallas, TX 75235.

Previous reports have demonstrated that subcutaneous administration of luteinizing hormone releasing-hormone (LRH) can facilitate lordosis behavior in estrogen-primed ovariectomized (OVX) and ovariectomized-hypophysectomized rats. In addition, microinfusion of 50 ng of LRH into the medial preoptic area (MPOA) or arcuate nucleus, but not into the lateral hypothalamic area or cerebral cortex can potentiate lordosis behavior in OVX estrone-primed rats.

Twelve OVX rats were implanted with 23 guage stainless steel cannulas in the midbrain central grey region under ether anesthesia. Each rat, which had demonstrated consistently high sexual receptivity with estrone-progesterone, was primed with 200 µg estrone sc. Forty-eight hrs later the animals were tested for preinfusion lordosis behavior (PRE) by mating with a sexually vigorous male for 15 mounts. Subsequently the rats were anesthetized with ether and infused with either 50 ng of LRH or 0.9% NaCl through a 30 guage stainless steel infusing cannula. The infusate volume of 0.5 µl was delivered over 60 seconds. One hour and 45 min later each rat was reintroduced into the mating arena and received 15 mounts (POST mating). Of 12 rats, 10 had higher lordosis-to-mount ratios (L/M) following LRH administration than with control. Luteinizing hormone-releasing hormone infusion significantly increased the ΔL/M (POST-PRE) over NaCl infusion alone: ΔL/M LRH-X=.594 SE=.081, ΔL/M NaCl-X=.30 SE=.079, $p < .002$.

Behaviorally active sites to LRH infusion within the hypothalamus correspond to regions of high LRH content. Since midbrain central grey receives afferents from MPOA and contains specific estrogen binding receptors, it may represent an extrahypothalamic locus for LRH potentiation of sexual behavior. These results support such an hypothesis. Further investigation will elucidate the physiologic role of this region in sex behavior, and its relationship to the previously demonstrated hypothalamic loci.

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1145 SEROTONIN RECEPTOR ANTAGONISM: SIMILARITIES AND DISSIMILARITIES TO THE ACTIONS OF PROGESTERONE ON THE SEXUAL BEHAVIOR OF THE FEMALE RAT. Jorge F. Rodriguez-Sierra* and Gary A. Davis* (SPON: A. I. Leshner). Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, Wisconsin 53706.

The reduction of serotonergic activity has been postulated as a possible mode of action of progesterone (P) in the facilitation of sexual behavior in estrogen-primed rats (Meyerson, 1964; Acta Physiol. Scand. 248). This initial facilitation is followed by a period of refractoriness to further induction of lordosis by additional P. This "biphasic" effect of P has been attributed by some workers to interference with estrogen priming.

The serotonin receptor antagonist Methysergide (MS) is known to facilitate lordosis. This study tested whether MS also exerts a biphasic action similar to P. Ovariectomized rats pretreated with estradiol benzoate (EB, 10 ug/Kg) were used in all experiments. Ten mounts by a male were allowed to all rats at 44, 48, 68, and 72 hours after EB. Lordosis quotients (LQ, lordoses/mounts x 100) were calculated as a measure of sexual responsiveness.

Treatment with MS 42 hours after EB gave rise to lordotic responding at 2 hr (LQ=60) and 6 hr (LQ=35) later, while vehicle injections had no effect. A second injection of MS at 68 hours was ineffective in MS-pretreated rats, while vehicle controls responded to MS at this time (LQ=66). Studies with P gave results similar to MS.

Despite this similarity, we have found that P facilitates sexual behavior in rats inhibited by MS (LQ=85). Similarly, MS facilitates lordosis in rats inhibited by P (LQ=80).

In order to determine if hypersensitivity of serotonin receptors could account for the "biphasic" action of MS, the response of MS-treated rats to the serotonin receptor agonists Quipazine and 5-Methoxy-O Dimethyltryptamine is being determined.

These results argue against the possibility that the inhibitory action of P (or MS) is due to an interference with estrogen priming. Furthermore, they suggest that the facilitatory action of progesterone involves more than a serotonergic mechanism.

(Supported by NIH #5 P40 RR 000167-16)

1146 LOCAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING MICROINJECTION OF β-ENDORPHIN INTO THE PERIAQUEDUCTAL GRAY OF THE RAT. Osamu Sakurada*, Yasuko F. Jacquet and Louis Sokoloff. (SPON: G. Maloney). Laboratory of Cerebral Metabolism, NIMH, Bethesda, Md. 20014, and NY State Res. Institute for Neurochem., Ward's Island, NY 10035.

Recent reports have indicated that β-LPH(61-91) (also called β-endorphin, or the C-fragment of β-LPH), when microinjected into rat brain, results in sedation, catatonia and analgesia (Science, 194: 632, 1976). In the present study, we used the recently developed autoradiographic [^{14}C]deoxyglucose method, which measures rates of local cerebral glucose utilization (LCGU), to investigate possible changes in the functional activity of various brain regions which might be correlated with the behavioral state induced by β-endorphin microinjection into the periaqueductal gray (PAG) of the unanesthetized rat. Experimental rats with chronically-implanted cannulae in the PAG were microinjected with 4-8 µg of β-endorphin into bilateral sites of the caudal periaqueductal gray. Control rats with chronically-implanted cannulae in similar sites were injected with an equivalent volume of the vehicle (i.e., physiological saline) alone. The results revealed a tendency for generally reduced rates of local cerebral glucose utilization in the β-endorphin-injected group compared to the saline-control group, but only the medial and lateral geniculate bodies exhibited statistically significant reductions at the $p < 0.05$ level. Other than these structures no regions were found to be specifically affected at the present level of resolution possible with the method.

1147 RADIOFREQUENCY LESIONS IN THE ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) BLOCK THE STEROID-INDUCED GONADOTROPIN SURGE. W.K. Samson* and S.M. McCann. Dept. of Physiology, Univ. of Texas Health Science Center at Dallas, Southwestern Med. Sch., Dallas, TX 75235.

Luteinizing Hormone Releasing Hormone (LRH) has been localized in the median eminence (ME) and OVLT of rats. In order to determine the significance of the rostral LRH concentration, radiofrequency lesions were placed in the OVLT of ovariectomized rats. Three days later, the rats received 5µg estradiol benzoate (E) in 50 µl corn oil subcutaneously at 10 a.m., followed two days later by 2mg progesterone (P) in .2ml corn oil subcutaneously at 10 a.m. Blood samples were collected via the jugular vein prior to lesioning, E injection, P injection, and six hours after P injection. Rats were decapitated eight hours after P, trunk bloods and pituitaries collected, and brains removed for histology and assay of LRH content by radioimmunoassay (RIA). Serum values of gonadotropins were determined by RIA. Rats with lesions restricted to the OVLT (n=12) completely lacked the steroid-induced FSH and LH surge seen in controls (n=14), operated controls (n=12), and sham controls (electrode placement but no lesion, n=12) ($p<.001$). Medial septal lesions (n=9) of the same size failed to block the steroid-induced gonadotropin surge. Pituitary content of gonadotropins was significantly greater in the OVLT lesioned rats than in all others. Brains were divided into three portions, anterior (including OVLT), posterior (including ME), and an intermediate zone. In OVLT lesioned rats, the tissue levels of LRH in all three zones were significantly reduced compared to all other groups. These results demonstrate that the rostral LRH containing elements are necessary for the positive feedback effect of gonadal steroids, and that the ME and intermediate zone LRH content is decreased by removal of the OVLT, suggesting the validity of the axonal transport theory for LRH delivery to the ME.

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1148 A THYROTROPIN RELEASING HORMONE-LIKE MATERIAL IN THE RAT RETINA: CHANGES DUE TO ENVIRONMENTAL LIGHTING. James M. Schaeffer*, Michael J. Brownstein and Julius Axelrod (SPON: D. Dresher) Lab. Clin. Sci., NIMH, NIMH, Bethesda, Md. 20014

Thyrotropin releasing hormone (TRH) is present in many brain regions in addition to the hypothalamus. We have shown that TRH-like material (measured by radioimmunoassay) is present in the rat retina. Bioassays have confirmed the presence of TSH-releasing activity in retinal extracts. In addition the immunoreactive compound in the retinal extract co-chromatographed with authentic TRH on a Bio-gel P-2 column and was inactivated by pyroglutamate amino peptidase and serum peptidases. The concentration of retinal TRH is high during the daytime (200-300 pg/retina) and low at night (50-100 pg/retina). Rats housed in continuous darkness and killed at 1400 hours had low levels of TRH and conversely, rats housed under continual light and killed during night time had high levels of TRH. This data shows that changes in TRH concentration are dependent upon environmental light rather than an internal rhythm. Injection of rats with cyclohexamide (20 mg/kg) at the time the lights are turned on, prevents the light induced rise in TRH, suggesting that protein synthesis is necessary for the daytime increase. The function of TRH in the visual system is being studied.

1149 PITUITARY CATECHOLAMINES IN THE RAT: EFFECT OF PERIPHERALLY AND CENTRALLY ADMINISTERED 6-HYDROXY-DOPAMINE. W. J. Shoemaker, V. Andersson*, and F. E. Bloom. The Salk Inst., La Jolla, CA 92037.

Although the pituitary gland is known to contain both norepinephrine (NE) and dopamine (DA), only recently has the origin of these substances been investigated. The present experiments sought to determine quantitatively the proportion of each catecholamine (CA) contributed to the pituitary by peripheral sympathetic sources and CNS sources, and to establish the susceptibility of the innervation to 6-hydroxydopamine (6-OHDA). NE and DA determinations were performed by a modification of the radio-enzyme assay on freshly dissected anterior and neuro-intermediate lobes. In some experiments the ocular iris was also assayed; all samples were normalized for protein content. In addition, some animals were processed for fluorescence microscopy using a modified glyoxylic acid perfusion system. Albino male adult rats given either i.p. or intravenous (i.v.) doses of 6-OHDA (100 mg/kg:1 dose/week x 4 weeks) failed to show any loss of NE or DA from either pituitary lobe when compared to either NaBr injected or uninjected controls. The biochemical measurements were confirmed by fluorescence microscopy; 6-OHDA-treated rat pituitaries are indistinquishable from normals. The insensitivity of the pituitary to i.v. 6-OHDA occurs in animals that have iris NE depleted > 80% by the drug. The apparent discrepancy with previous reports (Bjorklund et al., Brain Res. 51: 171, 1973) of denervation of neuro-intermediate lobe blood vessels by surgical sympathectomy has a likely explanation: The loss of NE incurred by destruction of fibers to some blood vessels may be too little to detect by biochemical assay. In an attempt to destroy the CA fibers from CNS sources, rats were given 2 doses (200µg each) of 6-OHDA, 24 hrs. apart, either by the intracisternal route or via permanently implanted cannula in the 3rd ventricle. In both preparations no loss of DA or NE from either lobe could be detected (e.g., DA of neuro-intermediate lobe: cannula 6-OHDA= 18.6; NaBr=15.2; uninjected=22.4 ng/mg prot.). These results are compatible both with our previous demonstration that most diencephalic DA-containing cells are resistant to 6-OHDA, and, with other reports that these same hypothalamic cells are the origin of most pituitary catecholamines.

(Supported by a grant from the Rockefeller Foundation.)

1150 ANGIOTENSIN INVOLVEMENT IN THE OSMOTIC CONTROL OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM. Celia D. Sladek and Robert J. Joynt*. Depts. Neurology and Anatomy, Univ. Rochester School of Medicine, Rochester, N.Y. 14642.

The organ cultured hypothalamo-neurohypophyseal system (HNS) has been used as an in vitro system for studying control of vasopressin (AVP) release. Each of these organotypic explants includes the supraoptic nucleus, median eminence, and neural lobe. The presence of viable supraoptic neurons in HNS explants maintained up to 9 days in culture has been confirmed by evaluating the histological and neurosecretory characteristics of the preparations. The HNS releases AVP at a constant rate in vitro and remains responsive to cholinergic and osmotic stimulation of AVP release. Additionally, angiotensin II is a potent stimulant for AVP release. Addition of angiotensin II to the culture medium at 10^{-6} or 10^{-5}M resulted in 3.8 and 4-fold increases in AVP release respectively ($p<.01$). Addition of saralasin acetate (P113), an angiotensin antagonist, blocked the response to angiotensin without significantly altering the basal rate of AVP release. Saralasin also blocked osmotically induced stimulation of AVP release. Increasing the osmolality of the culture medium from 285 to 300 mosmol/liter by the addition of NaCl resulted in a 3-fold increase in AVP release ($p<.01$). This was not observed when saralasin and NaCl were added concurrently. These data confirm previous observations of angiotensin stimulation of AVP release and suggest that angiotensin may be involved in the osmotic control of AVP release. Further experiments are required to elucidate the nature of this involvement.

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1151 MEDIAL PREOPTIC AREA AND STIMULATION OF NEST BUILDING IN MICE. Burton M. Slotnick, Aldair F. Simoes-Fontes*, and Jose C. Simoes Fontes*. Psychology Dept., The American University, Washington, DC 20016.

Individually housed virgin female CF-1 mice were allowed unlimited access to cotton thread nesting material for 21 days prior to experimental manipulations.

Experiment 1 demonstrated that 50-60 mg pellets of subcutaneous progesterone resulted in a 5-10 increase in nest building behavior. This effect could be reversed by removal of the progesterone pellet.

In Experiment 2 progesterone was implanted into various hypothalamic sites in each of 26 mice. The implant consisted of a 29-gauge cannula with the cross sectional opening filled with crystaline progesterone. Four mice showed a dramatic increase in nest building within 4 days after implantation. Histological examination revealed that the implants in each of these mice were located in the medial preoptic area. Mice with implants just lateral or dorsal to the medial preoptic area showed a similar but delayed (5-7 days) increase in nesting. The remaining mice, with implants in the region of the paraventricular nucleus and ventromedial hypothalamus, showed no change in nest building. Implants in the third ventricle were without effect.

In Experiment 3 the effects of subcutaneous progesterone on nest building of mice with medial preoptic lesions were examined. As compared to sham lesioned controls, small lesions confined to the medial portion of the medial preoptic area effectively blocked the increase in nest building by subcutaneous progesterone.

Our results suggest that the medial preoptic area mediates the induction of nest building behavior by progesterone in mice.

1152 CYCLIC NUCLEOTIDES IN NEUROENDOCRINE FUNCTION: THE DOPAMINE AND CHLORPROMAZINE. M. Smyser* and Y. Clement-Cormier*, (SPON: R. Peterson). Dept. Neurobiology and Anatomy, Univ. of Tex. Md. Sch. at Houston, Houston, Tex. 77025.

The distribution of dopamine within the median eminence correlates well with the distribution of some releasing hormones associated with this area. Furthermore, dopamine has long been know capable of facilitating hormone release through an effect that has been localized in the median eminence. This paper reports the occurrence of a dopamine stimulated adenylate cyclase in the median eminence. A half-maximal increase in enzyme activity was observed with 5 μM dopamine. The β-adrenergic agonists l-isoproterenol had no significant effect on adenylate cyclase activity with concentrations as high as 1000 μ M. Apomorphine, known to mimic the pharmacological and physiological effects of dopamine, stimulated adenylate cyclase activity in the median eminence. Several different classes of drugs effective in the treatment of schizophrenia and with well known endocrinological side effects were found to be potent inhibitors of the stimulation by dopamine of the enzyme. The phenothiazine, chlorpromazine was found to be a competitive inhibitor of adenylate cyclase with respect to dopamine. The calculated inhibition constant (Ki) for chlorpromazine was 1×10^{-7} M whereas that for an even more potent phenothiazine fluphenazine was 7.5×10^{-9} M. These studies implicate a role for dopamine-sensitive adenylate cyclase in neuroendocrine function.

These results in the median eminence encouraged us to investigate the action of dopamine and chlorpromazine on adenylate cyclase in the pituitary. Evidence exists showing that certain pharmacologic agents including the phenothiazines can increase prolactin release from the pituitary. In addition, dopamine has been shown to inhibit the action of the phenothiazines on prolactin release. Using homogenates of a cloned pituitary tumor cell line (GH_3/C_{14}) which releases prolactin and growth hormone, dopamine was found to be ineffective in stimulating adenylate cyclase at concentrations up to 3×10^{-4} M. However, chlorpromazine at concentrations as low as 10^{-7} M resulted in significant stimulation of adenylate cyclase. A half-maximal increase in activity of the GH_3/C_{14} adenylate cyclase occurred with 1×10^{-6} M. In addition, the chlorpromazine derivatives 7-methoxychlorpromazine, 7-hydroxychlorpromazine and 8-hydroxychlorpromazine were found to mimic the stimulating action on adenylate cyclase whereas chlorpromazine-5-N, dioxide was virtually ineffective. These results considered with other data suggest that hyperprolactinemia resulting as an effect of phenothiazine treatment may be attributable to a direct action of these drugs to increase adenylate cyclase activity in prolactin containing cells of the anterior pituitary rather than through an action on dopamine-sensitive cyclase in this area. (Supported in part by grants from the Pharmaceutical Manufacturer's Associaton and Upjohn.)

1153 THYROTROPIN RELEASING HORMONE BLOCKS GROWTH HORMONE RELEASE INDUCED BY TRYPTOPHAN IN THE RAT. Eliot Spindel*, Michael Arnold*, Richard Wurtman and John Fernstrom (SPON: Walter A. Rosenblith) Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

Thyrotropin releasing hormone (TRH) has been shown to block the increases in serum growth hormone (GH) caused by morphine and pentabarbitol (Brown and Vale Endo. 97:1151, 1975). We now report that TRH also blocks the increase caused by the amino acid tryptophan. Injection of tryptophan (200 mg/Kg i.p.) into gentled Wistar rats at a time of day when we observe serum GH levels to be low (11:00 AM) caused a two to five fold elevation in GH concentration after sixty minutes. This increase in GH was completely blocked if TRH was injected (1 μg/Kg s.c.) 30 minutes before sacrifice. In one experiment, TRH alone caused a significant decrease in GH. Since tryptophan administration specifically enhances brain serotonin formation and release, our data provide additional evidence that a serotoninergic synapse influences GH release and suggests that TRH acts to inhibit the secretion of GH induced by activating this serotoninergic synapse. The locus of this inhibitory action of TRH may be within the brain.

	Serum GH (ng/ml)		
Vehicle	Tryptophan	TRH	TRH + Tryptophan
37 ± 10*	202 ± 59	24 ± 7*	24 ± 5*
(17)	(18)	(11)	(18)

Mean ± SEM. Number of animals in parentheses. Data represents 3 experiments pooled. *$p < .05$ as compared to tryptophan group by Newman-Keuls test.

(Supported in part by USPHS grant AM-14228 & MH25857. Eliot Spindel is a NIMH trainee, grant MH-13449.)

1154 INHIBITION BY VENTROMEDIAL HYPOTHALAMIC DEAFFERENTATION OF 2-DEOXYGLUCOSE-AND IMMOBILIZATION-INDUCED RELEASE OF CATECHOLAMINES AND CORTICOSTERONE IN AWAKE RATS. C.L. Sun*, N.B. Thoa* and I.J. Kopin, (SPON: R.W. Colburn). Lab. of Clin. Sci., NIMH, Bethesda MD 20014.

Stressful stimuli evoke release of catecholamines (CA) and corticosterone (CX) by activation of the sympathetic adrenal medullary system and the hypothalamo-pituitary-adrenal axis. The afferent pathways by which they produce this activation seem to depend on the nature of the stimulus. The response to certain stresses depend on the integrity of the neural input into the medial basal hypothalamus whereas others (ether, insulin) do not (Makara et al., J. Endocrinol. 44: 187, 1969; Makara et al., J. Endocrinol. 47: 411, 1970). In the present study, the effects of 2-deoxy-glucose (2DG) were compared with immobilization (IMO) stress. Deafferentation (DX) of ventromedial hypothalamus (VMH) was performed in male Sprague-Dawley rats (450-550 gram). After 5 days, cannulae were implanted in their tail arteries for collection of blood. The next day, blood samples (0.5 ml) were obtained between 9:30-10:00 AM and 2DG (500 mg/kg, I.P.) administered or the animals immobilized and blood samples obtained after 15 and 45 minutes. Plasma norepinephrine (NE) and epinephrine (E) were assayed by a radioenzymatic method and CX by the micro-flurometry. After the experiments, the animals were decapitated and DX confirmed histologically. Plasma levels of CA and CX were similiar in sham and successfully operated undisturbed rats (NE, 350 pg/ml; E, 160 pg/ml; CX, 100 ng/ml). During IMO or after 2DG in sham-operated rats plasma levels of E were similiarly increased (10-fold and 12-fold, respectively) but NE levels were increased to a greater extent after IMO (4-fold) than after 2DG (2-fold). CX levels were similiarly increased with both stresses (4-fold). DX of the VMH resulted in a greater than 50% decrease in the response of plasma E to both IMO and 2DG. The response of NE and CX to IMO were also diminished to about 1/2, but there were no significant increases in plasma NE or CX after 2DG in the operated animals.

These results suggest that the adrenal medullary response to IMO and 2DG, indicated by the increase in plasma E, are partially mediated from sites outside the VMH, but that a significant response is possible when after DX. The effect of DX on the sympathetic (SYM) and adrenal cortical (AC) responses, indicated by plasma levels of NE and CX, vary with the nature of the stress. DX of the VMH almost completely blocks the SYM and AC responses to 2DG, but only partially blocks the responses to IMO. Thus the SYM and AC responses to 2DG appear to be mediated mainly by activation of sites outside the VMH while those of IMO are only partially controlled from such sites.

1155 MEASUREMENT OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN BLOOD OF NORMAL RATS. Gloria Shaffer Tannenbaum*, Jacques Epelbaum*, Joseph B. Martin and Paul Brazeau*. Dept. of Neurology, McGill University, Montreal, Quebec H3G 1A4.

Somatostatin (SRIF), a tetradecapeptide originally isolated from the hypothalamus, has subsequently been localized in several peripheral tissues including gastrointestinal tract and pancreas. However, its role in normal physiology remains unclear. Progress in this respect has been limited by the inability to measure SRIF in biological fluids. The aim of the present study was to develop a technique for the measurement of SRIF in blood.

Sequential blood samples were obtained every 15 min for periods of 6h from freely-moving male rats bearing chronic venous cannulae. Each blood sample (0.45 cc) was rapidly withdrawn and added to a centrifuge tube containing Trasylol (400 K.I.U.) to inhibit enzymatic degradation. Following centrifugation, plasma was immediately extracted in an equal volume of 2N acetic acid, and frozen at -20°C until subsequent assay. All samples were assayed within 3 days. Plasma SRIF-immunoreactivity was determined by a sensitive, specific radioimmunoassay (Brain Res. 1977, in press). Plasma growth hormone (GH) levels were simultaneously monitored in all animals.

Preliminary studies revealed that SRIF-deactivated plasma (plasma left at 20°C for 24h and then extracted with acetic acid) exhibited significant displacement of binding (ranging from 21-46%) in our assay system. In order to counteract this non-specific effect of plasma, all subsequent determinations of plasma SRIF levels were obtained from standard curves containing deactivated plasma from each sampled animal. These values are reported as somatostatin-like immunoreactivity (SLI). Recovery of 1000 pg synthetic SRIF added to deactivated rat plasma averaged 85% when analysed in this manner. The half-life of SRIF in blood, determined by i.v. injection of synthetic SRIF, was found to be less than 4 minutes. The 6-h secretory profiles, obtained in 7 rats, were characterized by 3-5 intermittent bursts of SLI rising from undetectable (<96 pg SLI/ml) to peak values of 384-4800 pg SLI/ml. An increased frequency of SLI bursts was observed during trough periods of GH secretion. Administration of urethane (125 mg/100 g b.w. i.p.) to 5 rats, which suppressed GH to undetectable levels, resulted in a significant rise in SLI levels (mean 1.5h post-urethane versus mean 1.5h prior to urethane was 1758 ± 381 vs 721 ± 176 pg SLI/ml, $p < .02$).

These results suggest that SLI can be measured in blood. Precise estimation of SRIF in blood is difficult because of non-specific plasma effects. Urethane anaesthesia appears to suppress GH secretion by release of endogenous SRIF.

1156 PASSIVE IMMUNIZATION WITH ANTISERUM TO SOMATOSTATIN (AS-SS): EFFECTS ON THE DYNAMICS OF GROWTH HORMONE (GH), PROLACTIN (PRL), AND THYROID STIMULATING HORMONE (TSH) SECRETION IN CANNULATED RATS. L.Cass Terry, Jacques Epelbaum*, Paul Brazeau*, and Joseph B. Martin. Dept. Neurology, McGill Univ., Montreal, Quebec.

Somatostatin (SRIF), a tetradecapeptide originally isolated from the hypothalamus, is reported to inhibit the release of GH, TSH, and (in some reports) PRL. Passive immunization with AS-SS has been reported to result in inhibition of stress-induced GH suppression and augmentation of the TSH response to cold or TRF (thyrotropin releasing factor). However, there is little information that describes the effects of AS-SS on the dynamics of GH, PRL and TSH secretion in the undisturbed, basal state.

Chronically cannulated, male Sprague-Dawley rats were kept on a 12:12h light-dark cycle and housed individually in isolation boxes to reduce environmental stimuli. Rats received 1 ml of either AS-SS or normal sheep serum (NSS) iv at 0900h. Blood samples (0.4 ml) were removed every 15 minutes from 1000 to 1600h. Each animal was sampled for a similar period two days before receiving AS-SS or NSS which allowed two sets of controls; NSS and baseline (BL). Plasma GH, PRL, and TSH were measured by radioimmunoassay kits supplied by the NIAMDD.

Mean values of all samples in AS-SS treated rats over the six hour period demonstrated a significant elevation of TSH ($P<.001$), but not of GH or PRL. Analysis of episodic GH secretion revealed no change in the frequency of bursts. There was a significant elevation of GH trough levels ($P<.005$) in AS-SS rats, but no change in peak values. There was no discernible effect on episodic secretion of TSH or PRL. The binding capacity of serum from AS-SS animals was determined at 1000 and 1600h. Both samples exhibited high capacity to bind SRIF but there was a significant reduction in binding capacity at 1600h ($P<.05$) compared to 1000h.

Preliminary evidence indicates that electrical stimulation in the medial forebrain-lateral hypothalamic region of AS-SS treated rats results in GH release and implies the existence of a GH-releasing factor. These results suggest that SRIF exerts a tonic inhibitory effect on basal TSH and GH secretion. Thus, AS-SS elevates baseline levels of GH without affecting its pulsatile nature. The use of AS-SS to suppress SRIF effects may permit unmasking of GRF secretion.

(Supported by the Medical Research Council of Canada).

1157 THE EFFECT OF CASTRATION ON HYPOTHALAMIC SEROTONERGIC NEURONS. L. van de Kar*, Jon Levine*, and L.S. Van Orden III, The Tox. Ctr., Depts. of Pharmacology and Psychiatry, Univ. of Iowa, Iowa City, IA 52242.

Several studies (Ladoski and Noronha 1974, Fuxe, and Hökfelt 1974) have suggested a regulatory role for serotonin in the LH secretory system. This study was conducted to investigate a possible interaction between serotonergic neurons in the hypothalamus and the LH secretory system. Using a radioisotopic assay for serotonin (Saavedra et al., 1973), the steady state level of serotonin in several hypothalamic nuclei of male rats was increased when measured 7 days after castration. This effect was reversed by the administration of testosterone propionate. The increase in serotonin content could be due to increased synthesis, decreased degradation, decreased release of serotonin or any combination of these parameters. In order to elucidate the mechanism of increased serotonin content, male rats were injected with pargyline-HCl (75 mg/kg i.p.), a monoamine oxidase inhibitor, at 10, 20, or 30 minutes before decapitation and the rate of accumulation of serotonin in hypothalamic nuclei was examined. Any accumulation of serotonin would be due to continuing synthesis of neurotransmitter when degradation by MAO is inhibited. The rate of serotonin accumulation was markedly decreased in hypothalamic nuclei from castrated rats and returned to normal in castrated rats which were maintained on testosterone propionate. These results suggest that the increased serotonin content of hypothalamic nuclei after castration may be a result of MAO inhibition and initial MAO levels are important in interpretation of results.

	Sham		Castrated		Castrated + Testosterone (5 mg)	
	5HT levels (ng/mg protein)	5HT turnover (ng/mg prot/hr)	5HT levels (ng/mg protein)	5HT turnover (ng/mg prot/hr)	5HT levels (ng/mg protein)	5HT turnover (ng/mg prot/hr)
MPOa	12.2± 3.8	59.9	17.7± 1.8	0.0	9.0± 2.4**	23.4
NCS	40.2±23.0	93.0	95.2±23.7	0.0	14.7± 3.1**	86.4
NHA	18.1± 6.4	30.1	21.7± 3.3	22.3	7.3± 2.1**	60.6
NA	33.5±13.4	27.6	40.2± 6.0	9.1	10.6± 1.4**	62.5
NVM	11.7± 5.1	37.3	28.1± 4.7*	0.0	6.7± 1.5**	47.8
MDH	11.0± 3.4	22.3	21.5± 3.0*	36.7	8.4± 3.3**	62.2
MFB	6.2± 2.9	64.9	26.9± 6.4*	17.0	13.4± 5.9	52.8
Caud.	3.5± 1.0	37.2	30.4± 5.6*	0.0	4.9± 1.1**	69.9

*Significant difference between the turnover of sham and castrated $P<.05$.
**Significant difference between the turnover of castrated and castrated + testosterone (5 mg) $P<.05$.

1158 ELEVATED BLOOD PRESSURE AFTER PINEALECTOMY IN THE RAT. G. M. Vaughan*, R. A. Becker* and J. P. Allen. Dept of Medicine, The Univ. of Texas Health Science Center, San Antonio, TX 78284

The pineal gland may be involved in regulation of blood pressure. To test this hypothesis, the effect of pinealectomy on blood pressure was studied in 35 to 123 day old male Sprague-Dawley rats. Systolic blood pressure (BP) and pulse rate were detected by the onset of Korotkoff sounds recorded distally on the tail, and superimposed on the pressure record obtained by slowly deflating a proximally positioned pneumatic cuff.

In the first experiment, pinealectomy (Px) or sham (ShPx) was performed on day 43 and blood pressure measured over the following 20 days. There was no significant difference in mean BP in the Px compared to ShPx groups. Following administration of 1% NaCl as sole fluid source from day 96 to 123, BP was not significantly different in the two groups. However, Px rats drank 65 ± 3.2, whereas ShPx rats ingested 51.7 ± 5.1 mg/day ($p<0.05$), with no difference in body weight. There was no change in pulse rate after the surgical procedures.

In the second experiment, Px or ShPx was performed on day 55 of life. Ten to 20 days after surgery, there was greater rise ($p<0.05$) in the mean BP in the Px group (14.4 ± 2.3 mm Hg) compared to that following ShPx (6.9 ± 2.3 mm Hg). This difference continued into the period when 1% saline was used as sole fluid source (days 96 to 123). During this time 5/6 Px rats averaged a BP $\geq$ 150 mm Hg (range 141 - 171), whereas only 1/7 ShPx rats averaged 150 (range 129 - 150). Mean BP was significantly higher ($P<0.05$) in Px rats compared to ShPx. However, neither saline intake nor pulse was significantly different between the groups. These data confirm that pinealectomy (on day 55) may result in mild hypertension in the rat. Further, this is not primarily due to increased heart rate or saline intake.

1159 EMBRYONIC ESTRADIOL RECEPTORS FROM MOUSE AND RAT BRAIN. Christine C. Vito* and Thomas O. Fox. Dept. of Neuropathology, Harvard Medical School, and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, Mass. 02115.

Sexual differentiation of the brain is influenced by sex steroids. Although the "critical period" of steroid responsiveness is thought to extend from late embryonic to early postnatal ages, the actual existence of sex steroid reception mechanisms in neonatal brain was established only recently.

The first demonstration of typical 4S-5S estradiol-binding macromolecules from neonatal mouse brains was achieved by fractionation with DNA-cellulose(DC) affinity chromatography. We have now developed "affinity-exchange" chromatography which allows us to qualitatively analyze specific estrogen receptors from embryonic brain cytosols free of the high capacity, low affinity estradiol-binding proteins. "Affinity-exchange" chromatography (1) exploits the affinity for DNA of estradiol-bound receptor to separate it from non-adhering macromolecules such as embryonic and neonatal estrogen-binding proteins and (2) permits the selective labeling of specific DC-adhering estrogen receptors with ^{3}H-estradiol via an exchange process at 30 C.

We present biochemical evidence that receptor-like, 4S-5S estradiol-binding macromolecules exist in embryonic hypothalamic-preoptic area(HPOA) and "brain"(whole brain minus HPOA) of both mice and rats. HPOA and "brain" cytosols were prepared from E16 (embryonic day 16) and E18 mouse and rat brains. Following an incubation with 200 nM non-radioactive estradiol, the macromolecular contents of each cytosol were chromatographed on a DC column and washed to remove the non-adhering proteins. Each DC column, containing the DNA-adhering proteins present in each cytosol, was subsequently loaded with ^{3}H-estradiol and incubated for 60 min at 30 C. After this "exchange," the now ^{3}H-labeled contents of each column were eluted and layered on sucrose gradients.

The resultant sedimentation profile obtained from each embryonic cytosol revealed receptor-like macromolecules with 4S-5S sedimentation rates. Other characteristics of these embryonic receptors, such as their apparent affinity for estradiol and DNA adherence, are also typical of cytosol estradiol receptors from neonatal and adult brain. The levels of estradiol receptor per mg of tissue appear to be less in E16 mouse HPOA than in adults. Also, the concentration of estradiol receptor in E16 and E18 mice and rats, as in neonates, appears to be similar in "brain" and HPOA whereas, in adults, the concentration is higher in HPOA.

1160 EFFECT OF ANTERIOR HYPOTHALAMIC CUTS AND ANTIBODY ADSORPTION OF LUTEINIZING HORMONE ON GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN DISCRETE REGIONS OF BRAIN. Cleatus J. Wallis* and William G. Luttge. Dept. of Neuroscience, Sch. Med., Univ. of Florida, Gainesville, FL 32601.

Glutamic acid decarboxylase (GAD) was measured in 14 brain regions of female rats 30 days after either an anterior knife cut (AC) placed just posterior to the suprachiasmatic nuclei or sham surgery (SC). In both intact and ovariectomized AC rats, GAD activity was significantly reduced in the arcuate nucleus ($p<0.01$), anterior hypothalamus ($p<0.05$) and ventromedial hypothalamus ($p<0.05$) as compared to SC controls. While GAD was not reduced in the nucleus interstitialis stria medullaris of ovariectomized AC animals it was reduced in intact AC animals, possibly due to high levels of estrogen as indicated by constant estrus vaginal smears and large uterine weights.

Ovariectomized rats were passively immunized by three daily tail vein injections of rabbit anti-rat LH antibody in a dose adequate to prevent ovulation in proestrus rats. Adsorption of LH resulted in reduction in GAD activity only in the ventromedial hypothalamus ($p<0.01$) and ventral tegmentum ($p<0.05$).

These results suggest that regional GAD activity may be modified by circulating levels of both protein and steroidal hormones.

1161 DISCRETE LESIONS OF SUPRACHIASMATIC PORTION OF PREOPTIC NUCLEUS OR SUPRACHIASMATIC NUCLEUS: INDUCTION OF CONSTANT ESTRUS AND CHANGES IN STEROID CONTROL OF GONADOTROPIN SECRETION. S. J. Wiegand*, E. Terasawa and W. E. Bridson*. Wisconsin Regional Primate Res. Ctr., Univ. Wisconsin, Madison 53706.

Large electrolytic lesions of the medial preoptic-anterior hypothalamic area (MPO-AH) or anterior hypothalamic deafferentation blocks ovulation and induces constant vaginal estrus in female rats. In the present study, the extent to which specific structures within MPO-AH must be damaged to induce this anovulatory syndrome has been established. Adult female Sprague-Dawley rats were housed under 14:10 light-dark cycle (light on 0500-1900) and animals showing regular 4 or 5 day cycles were selected. Small electrolytic lesions were placed in 4 loci within MPO-AH (Fig. 1), and the effect of these lesions on the vaginal estrous cycle observed for 4 weeks postoperatively. Experimental subjects evidencing disruption of normal estrous cyclicity were then ovariectomized, and effects of exogenous estrogen and progesterone on gonadotropin secretion examined. On days 15-19 post-ovariectomy blood samples were obtained daily at 1000 and 1700. Estradiol benzoate (5 µg) injection at 1200 on the second day of blood drawing was followed 48 hrs later by progesterone (1.5 mg). Plasma LH levels were determined by radioimmunoassay. Lesions A, B and C were effective in inducing constant estrus. Lesion D did not induce constant vaginal estrus, however, periods of prolonged diestrus were observed. At 1700, subsequent to progesterone injection, an LH surge was observed in control and sham-operated animals and in animals in lesion groups C (n=2) and D (n=6). The LH surge was not observed in animals with lesion A (n=5) or B (n=7). LH levels after estrogen but prior to progesterone injections were lower in groups A and B compared to controls. Thus, in addition to large lesions of the MPO-AH, small lesions restricted to the POSC or SC are sufficient to induce constant vaginal estrus. However, the destruction of the POSC alone, but not the SC alone, is capable of eliminating the LH surge in response to exogenously administered steroids, demonstrating that disruption of the LH surge and induction of constant estrus can be dissociated

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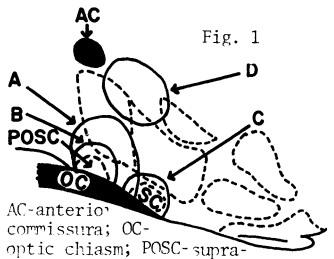

Fig. 1

AC-anterior commissura; OC-optic chiasm; POSC-suprachiasmatic portion of preoptic nucleus; SC-suprachiasmatic nucleus

1162 ELECTROPHYSIOLOGICAL EFFECTS OF CORTICOSTERONE AND 5α-DIHYDRO-CORTICOSTERONE IN THE PONTINE RETICULAR FORMATION. D.R. Williams, I. Kraulis* and B.O. Dubrovsky. Allan Memorial Institute, McGill University, Montreal.

Intravenous injections of corticosterone (B), (.75mg/.5cc/300gm) consistently increased (30-40% above baseline amplitude; onset lag: 3-5 min.; recovery: 10-15 min.) the amplitude of the sciatic evoked potential (EP) in the pontine reticular formation (RF) of the adrenalectomized rat whereas an identical dose of its reduced metabolite, 5α-dihydro-corticosterone (DB) caused a decrease (17-67% below baseline amplitude; onset lag: 2-8 min.; recovery: 10-15 min.). Control injections of 20% Cremophor-El in saline had no effect. These data show a greater potency and more rapid onset of steroidal action but otherwise confirm our previous observations with intraperitoneal injection (Neurosc. Abs. no.687, 1975; Neurosc. Abs. no.945, 1976) and strengthen the proposition that the responsiveness of the RF may be differentially affected by circulating levels of endogenous steroids. Since CNS excitatory and depressant steroids have long been known to oppose the central effects of one another (Woodbury, J. Pharmacol. 105, 27: 1952) we have looked for a possible interaction of B and DB on central sites. B was injected immediately after a DB induced decrease in EP was evident. The following responses were observed: (i) B caused an immediate increase in EP. (ii) B caused a transient increase in EP, followed by a marked decrease and prolonged recovery. (iii) B prolonged the recovery time of DB without affecting the amplitude of the EP. B was also found to increase EP's or components of EP's which were unresponsive to DB, indicating that the two steroids do not necessarily interact at the same sites. Preliminary studies at the neuronal level with multi-barrelled micropipettes for the electroiontophoretic release of steroids dissolved in ethanol suggest that there are B and DB responsive cells in the pontine RF and that these may be differentially responsive to B or DB. Available data also indicate that neurons in the dorsal regions of the RF (AP post. 6.5-7.5; ML lat. 2.0-2.2; DV H 6.5-7.5)* respond with an increase in their firing rate to electro-iontophoretically applied B while neurons more ventrally located (AP post. 6.5-7.5; ML lat. 2.0-2.2; DV H 7.5-9.0) respond with a decrease and suggest that site specificity in the neuronal response to B may exist in the pontine RF.

* A Stereotaxic Atlas of the Hooded Rat Brain
Pellegrino and Cushman 1966

1163 AN AUTORADIOGRAPHIC STUDY OF ^{3}H-ANDROGEN RETENTION IN THE BRAIN OF A SONGBIRD (FRINGILLA COELEBS). R.E. Zigmond, R. Detrick*, and D.W. Pfaff. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115 and Rockefeller University, New York, N.Y. 10021.

Previous workers have shown that there is an overlap between the distribution of androgen concentrating cells in the rat brain and areas of the brain from which implants of testosterone can elicit mating behavior. We studied the distribution of androgen-concentrating cells in the chaffinch (a songbird) to see whether in an animal with a different set of androgen-dependent behavior patterns (and presumably a different neural substrate for such behaviors) a different distribution of androgen-concentrating cells would be found. We previously reported that an area of the midbrain suspected to be involved in the control of vocal behavior contained a large number of androgen-concentrating cells (Zigmond et al., 179: 1005, 1973). We now report our finding of androgen-concentrating cells in other areas of the chaffinch brain suspected to be involved in the control of vocalization and other aspects of mating behavior. Four male birds were castrated and after one to three days they were injected with 0.4 µg of [^{3}H] testosterone (91 c/m mole) intramuscularly. An hour later the birds were killed, their brains quickly removed and frozen, and six micron coronal sections of the brain were cut at -19°C. The sections were placed on slides precoated with emulsion and exposed for about 5 months at 4°C. The autoradiograms were then developed and stained with cresyl violet and the distribution of labelled cells was analyzed quantitatively in over eighty sections. A labelled cell was defined as one which had at least five times the density of reduced silver grains over it as found in areas between cells. (Extensive background counts demonstrated that such a cluster of grains would almost never occur by chance alone.) Labelled cells were particularly concentrated in certain regions of the brain including the magnocellular nucleus of the anterior neostriatum, the caudal nucleus of the hyperstriatum ventralis, the periventricular area of the medial neostriatum, the medial preoptic area, the magnocellular periventricular nucleus of the hypothalamus, the medial posterior hypothalamic nucleus, the lateral septum, the intercollicular nucleus of the midbrain and the hypoglossal nucleus. Grain counting showed that the mean number of grains per cell varied in different brain regions.

The results indicate a substantial overlap between the distribution of androgen-concentrating cells and the distribution of neurons thought to be involved in the control of a number of androgen-dependent behaviors in the chaffinch.

NEUROETHOLOGY

1164 THE NEUROETHOLOGISTS HANDICAP: THE CONCEPTS OF FIXED ACTION PATTERNS AND BEHAVIORAL HIERARCHIES. K. Bellman*(Spons: W. Heiligenberg). Dept Psychology, University of California, San Diego, 92093.

The concept of fixed action pattern (FAP) was developed in order to describe the consistency of an animal's behavior. Though primarily a descriptive term, it possesses strong organizational connotations. These are apparent when the FAP is used as the "minimal unit" of behavior. It is what is being selected, maintained and sequenced with respect to other behaviors. A problem with this view is that when faced with competition between two FAPs, ethologists were forced to adopt the notion of behavioral hierarchies as a way of resolving the conflict. W.Davis (JCP,90:207-243,1974) defines behavioral hierarchies as "the organization of different and unrelated behavioral acts into an explicit priority sequence." Ever since these concepts have been formulated,Lorenz, Tinbergen and others have looked for their neural correlates. In so far as these concepts are inadequate,the neuroethologist's understanding of the neural substrates of behavior will be similarly deficient. The resolution of competition between partially incompatible actions is considered a major problem for a nervous system and a fundamental factor in the design of neural organizations. As a competition-resolving mechanism,the behavioral hierarchy is considered to be inadequate for several reasons. In order to test these criticisms,the competition-resolving capabilities of an animal known for FAPs was tested. Two species of lizards were placed in a series of conflict situations between two highly stereotypic behaviors: aggression and feeding. Results thus far show that both species of lizards when faced with conflict will not choose one behavior over the other as implied by behavioral hierarchies. Rather both species will modify the behavior patterns in several interesting ways. These modifications include timesharing or alternating between patterns and actual blending of postures into ones intermediate to the behaviors. The implication of timesharing and merging capabilities are discussed with respect to neural models of behavior.

1165 BEHAVIORAL ANALYSIS OF RANGE OF OBJECT DETECTION (ELECTROLOCATION) IN THE WEAKLY ELECTRIC FISH, APTERONOTUS ALBIFRONS. Rocco A. Bombardieri[1] and Albert S. Feng[2]. Neurobiol. Unit, Scripps Inst. Oceanography and Dept Neurosci., Sch. Med. UCSD, La Jolla, CA 92093

The 'respiration response', an unconditioned slowing of respiration in response to moving Plexiglas or aluminum rods, mediated by the electrosensory system, was used to measure the object detecting ability of Apteronotus albifrons (Apteronotidae, Gymnotoidei) in order to compare behavioral to physiological responses[3].

Respiration was recorded between an implanted electrode and a second electrode in the aquarium water, adjusted to 2K ohm-cm resistivity. Fish were held in a nylon mesh net in the center of a 27 X 121 X 27cm aquarium. All fish were blinded.

The strength of the respiration response is a function of object distance. The range of object detection is about 6 cm, which is greater than that reported for electroreceptors (2-2.5cm) but appears close to the range shown for cerebellar neurons (4-5cm)[3].

In an experiment in which responses to a 12mm metal rod were compared to those to a 19mm Plexiglas rod the smaller metal rod elicited stronger responses which is similar to results from electroreceptors where metal objects gave stronger responses[3].

In an experiment on object size a 3mm metal rod was compared to a 12mm rod and essentially similar responses were obtained. This may be contrasted to the electroreceptors where larger objects gave larger responses but is similar to the cerebellar neurons[3].

The significance of these data lies in their providing an opportunity to compare an animal's sensory performance from behavioral data to that from physiological recordings at two levels in the nervous system[3].

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[2]Pres. addr: Physiol. and Biophysics, Univ. Ill., Urbana, IL 61801.

[3]Bastian, J., The range of electrolocation: a comparison of electroreceptor responses and the responses of cerebellar neurons in a gymnotid fish, J. comp. Physiol., 108(1976) 193-210.

1166 EVOKED POTENTIALS IN THE BRAIN IN ELASMOBRANCHS AND SILUROIDS TO PHYSIOLOGICAL STIMULATION OF ELECTRORECEPTORS. Theodore H. Bullock. Dept. Neurosc. and Neurobiol. Unit, Scripps Inst. Oceanog., UCSD, La Jolla, CA 92093.

Averaged evoked potentials (AEP's) were recorded by metal semi-microelectrodes either manipulator-held in the exposed brain, or implanted. Quasi-homogeneous electric fields were created by constant current pulses or sine waves.

Sharks (Carcharhinus, Negaprion, Triakis) and the freshwater ray (Potamotrygon) were studied, extending the report of Platt et al., 1974, J. comp. Physiol. 95:323-355. A complex sequence of slow waves and spike bursts (averaged after passing through an inverter) depends for form and latency on brain locus (medulla, mesencephalon, telencephalon), current strength, polarity, orientation, sine wave frequency,and facilitation or antifacilitation from recent responses. Rays (6 genera) typically show longer latencies than sharks (5 genera). Both are typically longer than acoustic, shorter than photic latencies. AEP's show sensitivity down to 0.015 μv/cm in marine species (= 0.8 nA/cm^2). Potamotrygon in 70 Kohm·cm water is sensitive to <50 μv/cm (= 0.7 nA/cm^2). To sinusoidal stimuli the best response by a certain criterion is at 15-30 Hz in Carcharhinus, 5-15 Hz in Potamotrygon, but this depends on the stimulus regime and on the recording site.

Electroreception is now well known in a few catfish but it is not known how general this is among the diverse families of the large group of Siluroidea. I find a good AEP to weak electric stimuli in a species of each of these genera: Sorubim, Pseudoplatystoma, Goeldiella (Pimelodidae), Centrodoras, Oxydoras (Doradidae), Ancistrus (Loricariidae), Callichthys (Callichthyidae). All show responses in the mesencephalon, in the region of the torus semicircularis to electric fields of <2 mv/cm (70 Kohm·cm water) and often down to <0.15 mv/cm (= 2 nA/cm^2). Differences among the families are probable in discrimination of dipole orientation, position, and distance and of frequency of sinusoidal stimuli. (Aided by NSF, NIH, and by the Technische Hochschule, Darmstadt and the Natl. Inst. Amazonian Res., Manaus, Brazil.)

1167 EFFECT OF MIDBRAIN LESIONS ON FEMALE NEST-COO BEHAVIOR DURING THE BREEDING CYCLE. Jeffrey Cohen* and Mei F. Cheng* (SPON: R.L. Thompson). Inst. of Anim. Behav., Rutgers University, Newark, NJ 07102.

Nest-coo is a behavior pattern in which the ring dove assumes an oblique or squat position with its rump end up, flips its wings, and emits a coo-like sound. The performance of this behavior pattern by female ring doves coincides with the drastic growth of the ovarian follicle in response to male courtship, and it is estrogen dependent (Cheng,JCPP 83: 221, 234, 1973). The nucleus intercollicularis (ICo) region has been shown to be involved in the production of male song in a number of passerine species (Nottebohm, Stokes, and Leonard, JCN 165: 457, 1976). In the ring dove, the ICo region has been identified by autoradiographic methods as one that retains estrogen (Martinez-Vargas, Stumpf, and Sar, JCN 167: 83, 1976). Furthermore, local implantation of crystalline estrogen into this region elicits nest-coo behavior in the ovariectomized female ring dove (Cheng, unpub. data). We therefore made lesions in the ICo region to determine their possible effects on nest-coo behavior. Female ring doves of similar ovarian stage received either bilateral electrolytic lesions in the ICo region or sham lesions. Each female was then tested with a sexually active male (two hours, every third day) over an average of 27 days. The occurrence of various reproductive behaviors such as sexual crouch, copulation, and nest-coo were recorded. At the conclusion of testing, females were sacrificed to determine a) site of lesion, b) ovarian weight, oviduct weight, and follicle size. Some females with bilateral lesions of the ICo region failed to show nest-coo behavior. Other ICo lesioned females showed a significant increase in latency to nest-coo, compared to shams. Other reproductive behaviors (sexual crouch) were unaffected. The mean follicle size of ICo lesioned females did not change while that of sham animals showed a significant increase in response to male courtship. These data suggest the following: The ICo region is involved in the elicitation of the nest-coo and appears to exert some effect on the gonadal system. Alternatively the effect of ICo lesions on follicle size may suggest that behavior (nest-coo) itself has a direct effect on the female's own endocrine system.

1168 A ROLE FOR THE LARYNGEAL BRANCH OF CRANIAL NERVE X IN THE RELEASE CALL OF RANA PIPIENS. Carol Diakow and Alan DeFalco* Biology Department, Adelphi University, Garden City, New York 11530.

This study is one of a series on the neurobiology of reproductive behavior of the frog. It was designed to test for a role of the laryngeal nerve in the release call of Rana pipiens. Males and females were each tested 5 times by 30 sec of manual stimulation of the trunk and the number of release calls were recorded. Subjects then were subjected to bilateral or unilateral nerve section, or to sham operations, and retested 5 times. The mean of the mean number of pre operative release calls of the 29 males was 32.7. This was significantly higher than the 21.4 observed for the 26 females; therefore, the data for the males and females were handled separately. Results follow:

Mean ± s.d. Number of Release Calls in 30sec. (FEMALES)

	sham operation n=7	bilateral denervation n=7	right side denervation n=6	left side denervation n=6
PRE OP	21.2 ±10.5	21.0 ±8.4	24.0 ±8.5	21.4 ±12.6
POST OP	14.3 ±10.6	2.0 ±3.9	11.6 ±6.0	13.3 ±13.3

Mean ± s.d. Number of Release Calls in 30sec. (MALES)

	n=7	n=7	n=6	n=9
PRE OP	34.2 ±8.9	30.5 ±7.6	32.5 ±13.5	33.5 ±10.4
POST OP	30.9 ± 11.6	0.0	30.1 ±18.4	26.8 ±11.4

For sham operates, the pre and post op scores did not differ significantly. For each sex, analysis of variance indicates a significant difference among the post op scores, with the reduction in release calling after bilateral denervation being significantly lower than that for sham operates. Release call rate after unilateral denervation was not significantly different than that for sham operates. Based on these data, it is concluded that the laryngeal nerve must be intact, at least unilaterally, for Rana pipiens to maintain a normal rate of release calling.

1169 MERGING OF MODALITIES IN THE OPTIC TECTUM: INFRARED AND VISUAL INTEGRATION IN RATTLESNAKES. Peter H. Hartline, Michael S. Loop, and Leonard Kass. Neural and Behavioral Biology Program and Department of Physiology, University of Illinois, Urbana, IL 61801

In the superficial 500 μm of the optic tectum of Crotalus viridis, neurons are found which have only visual receptive fields (usually contralaterally driven). From 300-1200 μm, neurons driven by the contralateral infrared pit organ and neurons driven by either infrared or visual stimulation are found.

Most visual neurons respond best to introduction or movement of a dark object into a receptive field 5-25° across. Some have inhibitory areas, and some exhibit directional preference. Infrared neurons respond best to introduction or movement of a warm object into an excitatory receptive field 20-70° across the broadest axis. Response to onset of a sustained warm stimulus or removal of a cold one is a transient burst of impulses. Computer-scanned sensitivity contour plots show asymmetry of these fields. Some have inhibitory regions (also asymmetrical). Some infrared neurons exhibit directional motion preference.

Some bimodal neurons respond to either infrared or visual stimulation ("OR" units), and a receptive field could be found for separate stimulation of each modality. Others require concurrent visual and infrared stimulation ("AND" units), such as motion of the hand or a warm object in a lighted room.

Multimodal "OR" neurons have receptive fields whose locations are predictable from the multi-unit spatiotopic maps of the tectum for infrared and visual systems. These maps have similarly oriented axes, but important differences in magnification and in precise detail (Kass, Loop & Hartline, these abstracts). Consider a given tectal locus which receives input from different regions of space in the two sensory systems: a bimodal "OR" neuron at this tectal locus would have similarly disparate visual and infrared receptive fields. Evidently, bimodal neurons do not "correct" the spatial disparity between the infrared and visual maps.

These findings suggest that during development, retinal fibers spread out across the entire tectum to fill available sites, and that infrared neurons (whose composite receptive field is smaller than the composite visual field of an eye) similarly spread out to fill available sites in a different tectal layer. The mapping processes must be similar, but there is evidently no mechanism to bring infrared neurons representing one region of space to the same place where the corresponding visual neurons project or vice versa. Bimodal neurons take their input from a given tectal region, without correcting for the spatial disparity which exists between visual and infrared layers.

1170 ELECTRORECEPTION AND SPECIES RECOGNITION AMONG ELECTRIC FISH. Carl D. Hopkins. Dept. Ecology and Behavioral Biology; Univ. Minnesota; Minneapolis, Minn. 55455.

Gymnotoid and mormyriform electric fish produce and sense electric signals for object detection (electrolocation) and for social communication. Field studies of electric fish in Guyana, in Surinam, and in West Africa, show that the electric organ discharges (EODs) of sympatric species can be highly divergent. Those species with wave-like EODs tend to have divergent frequencies; those with pulse-like EODs tend toward divergent waveforms. Pulse EOD durations from fish within the genus, *Hypopomus* (Gymnotoidei) measure 0.8, 1.5, and 2.5 msec, for three species in Surinam; durations for those in the genus, *Brienomyrus* (Mormyridae) measure 0.3, 0.4, 0.6, 0.8, 1.2, 1.8, 2.5, and 8.0 msec, for the species in Gabon. Pulse durations appear to correlate both with preferred habitat, and with social spacing. Divergence of waveforms probably also occurs because coevolving species have needs for species recognition cues, and for channel privacy. While the species from these genera show a high degree of divergence, species from others are less diverse. Fourier analysis of pulse EODs reveals species-specific differences in the often complex power spectrum that are potentially significant physiologically in recognition.

Electrophysiological studies of electroreceptors among the *Hypopomus* species from Surinam confirm Bastian's findings (J. Comp. Physiol. 1976, 112:165) of two major tuberous electroreceptor types: pulse-marker units (short latency, single spike response to EOD) and burst-duration coders (longer latency, burst of spikes after EOD). Burst-duration coders have varied frequency responses: narrow band units are tuned to the peak frequency of the EOD spectrum; wide band units are tuned lower and more broadly, low pass units cut off at high, but not low frequencies (500 Hz). Pulse-marker units are also tuned to the peak frequency of the EOD spectrum. Each species has all four types, with differing frequency ranges correlating with EOD output.

Pulse-marker units and narrow-band burst-duration coders are thought to play a critical role in identification and recognition of species-specific EODs used in social communication.

1171 TELENCEPHALIC EFFERENT PATHWAYS AND FEEDING BEHAVIOR IN THE PIGEON. R.R. Levine* and H.P. Zeigler. Dept. Psych., Hunter College, CUNY, and Dept.Anim.Behav.,Am.Mus.Nat.Hist., New York, N.Y. 10024.

The present study examined the role of two extratelencephalic efferent pathways in the feeding behavior of the pigeon. The structures chosen for study, the occipitomesencephalic tract (OMT) and the septomesencephalic tract (SMT), arise in the archistriatum and hyperstriatum, respectively, and convey information to brain stem and spinal cord nuclei related to sensory systems (somatomotor and visual) involved in the exteroceptive control of feeding in this species. In addition, both tracts have been homologized with identified components of the mammalian pyramidal system.

Electrolytic lesions were placed in these tracts and their effects upon several measures of ingestive behaviors were examined. While there was no evidence for its direct involvement in drinking, OMT damage resulted in a reduced responsiveness to food which was manifested by periods of aphagia, hypophagia and decreased body weight. Furthermore, OMT birds evidenced a disruption of the consummatory response of pecking which was characterized by impairments in grasping (incomplete opening of the oral aperture) and pecking inaccuracy ("overshoots"). Finally, OMT damage temporarily abolished operant key-pecking reinforced with food. Resumption of key-pecking was marked by significant reductions in feeding and consummatory efficiency, which, in several cases, led to extinction of the key-pecking response. Lengthening the reinforcement intervals, permitting more feeding time, reinstated preoperative levels of key-pecking. However, all other behavioral measures showed persistent impairments. These findings indicate the OMT damage produces both consummatory (somatomotor) and "motivational" (responsiveness) deficits.

SMT lesions resulted in reduced food intake, but did not directly affect water intake. Unlike OMT birds, there were no decreases in responsiveness to food. Furthermore, there were no observable changes in the efficiency or accuracy of their feeding response and only transient reduction in operant responding. SMT birds, however, displayed inappropriate "sorting" behavior which suggests that the lesions may have affected visually controlled preference behavior.

OMT lesions produced feeding behavior deficits similar to those resulting from damage to central trigeminal structures previously implicated in the control of feeding behavior in the pigeon. In view of the anatomical connections between both sets of structures, these findings suggest that the OMT may be part of the pigeon's putative neural feeding system.

1172 EVOLUTION OF SOUND PRODUCTION IN A COCKROACH: STRUCTURAL BASIS AND BEHAVIORAL SIGNIFICANCE. Margaret C. Nelson[1] and Jean Fraser*[2], Dept. Neurobiology[1], Harvard Medical School, Boston, MA 02115, and Dept. Psychology[2], Brandeis Univ., Waltham, MA 02154.

In analyzing the mechanisms of evolutionary changes in behavior it is desirable to define both the role of the behavior in question and the neural/muscular elements involved in its control. We report an evolutionarily "new" behavior that is particularly amenable to such definition. The Madagascar cockroach, Gromphadorhina portentosa, produces audible hisses from a pair of modified abdominal spiracles that have lost their respiratory function. We have established that 4 types of hisses exist and that they serve a communicative purpose. We have also characterized morphological and physiological changes undergone by the modified spiracles during evolution, comparing them to serially homologous spiracles that retain a respiratory function.

The 4 types of hisses occur in differing behavioral contexts and differ in their acoustical characteristics. Adults and older nymphs of both sexes hiss in response to any sudden or disruptive stimulus. Adult males also hiss during courtship, copulation, and aggressive display. These 4 types of hisses (disturbance, courtship, copulatory and aggressive) differ acoustically from one another in absolute amplitude, amplitude envelope, and hiss-train characteristics. Thus, categorizing hisses either by behavioral context or by acoustical characteristics sorts hisses identically. In addition we have observed the effects of placing "muted" males, which are unable to hiss, in courtship or aggressive encounters. Muted males fail to copulate, although their courtship behavior appears normal. Muted males also lose a greater number of fights than predicted from their past performance. Thus several lines of evidence support the hypothesis that hissing serves as communication.

Because the modified spiracle is one in a population of serially homologous structures, one can determine which elements have undergone change and which have remained stable during evolution. Changes in the sound-production apparatus (4th spiracle) have occurred at several points. Morphological differences exist in the trachea, in the opener and closer muscles, and possibly in motoneurons, although the major branching patterns of the latter appear to have been conserved. The rhythmic relationship between ventilatory and spiracular motoneuron outputs also differs in the two classes of spiracles. Thus in this case it may be possible to specify in detail the cellular changes underlying the evolution of an ethologically significant behavior pattern. (Supported in part by NIH grant NS 11010 to Dr. J.G. Hildebrand and by NIH Training Grants NS 05731 and NS 07009.)

1173 TEGMENTAL INFLUENCES ON VOCAL PRODUCTION IN SQUIRREL MONKEYS. J. D. Newman, P. D. MacLean and R. E. Gelhard*. Behavioral Biology Branch, NICHD, NIH and Laboratory of Brain, Evolution and Behavior, NIMH, Bethesda, Md. 20014.

In testing the effects of various brain lesions on the display behavior of squirrel monkeys, abnormal vocalizations were observed following electrocoagulations placed in the diencephalic tegmentum. Spectrographic analysis of the vocalizations of such animals revealed changes ranging from the production of infantile sounds (otherwise found only in the first natal year) to an inability to utter certain elements of the vocal repetoire. The most notable effect was the persistent production of abnormal isolation peeps, including the addition of new harmonic components. The present findings indicate that the diencephalic tegmentum plays an important role in elaborating certain sounds of the squirrel monkey's vocal repetoire.

1174 SINGLE UNIT RECORDING FROM UNRESTRAINED CATS DURING NATURALLY-OCCURRING BITING ATTACK. C. A. Opsahl* (SPON: J. P. Flynn). Dept. Psychiatry, Yale Med. Sch., New Haven, Ct. 06508.

Extracellular recordings were made from 437 cells in five adult cats which spontaneously attacked mice. The cats were fed ad lib and thus did not usually eat the mice. Each cat was fitted with a microdrive assembly that permitted recordings to be made while the cat was unrestrained. The amplitude, duration, and waveform of the single unit activity suggested that the recordings were from cell bodies. Single unit activity was recorded from thalamus, hypothalamus (anterior, posterior, perifornical, far lateral, premammillary), zona incerta, fields of Forel, globus pallidus, amygdala, septum, anterior commissure, superior colliculus, central gray, midbrain reticular formation, and ventral tegmentum. As shown in Table 1, cells were found which increased baseline firing rates only during attack trials. Some cells were correlated with only one component of the behavior (e.g. viewing, pinioning, striking, or biting the mouse) whereas other cells were correlated with several components of the behavior. The specificity of each cell was determined by interspersing a large number of control trials among the trials when the mouse was presented. Sensory controls consisted of the presentation of visual, auditory, tactile, and olfactory stimuli, as well as trials when canned cat food was presented and consumed. Motor controls consisted of lifting the cat, passive and active head movements, leg retraction, and walking. In addition, a sixth cat which did not attack mice was recorded from under identical conditions. Of the 140 cells encountered in this cat, none changed firing rate to the presentation of a mouse. Single units that increased firing rates only during attack trials were found in two regions: the perifornical area of the lateral hypothalamus and the ventrolateral aspect of the midbrain central gray. Cells that increased firing frequency during feeding trials were located in perifornical LH and in the amygdala. It is suggested that these attack-related cells are involved in the integrated, directed behavior of the cat toward the mouse.

Table 1

Responsive only during attack on mouse	15
More Responsive during attack on mouse than during any other condition	13
Responsive only during feeding trials	4
Responsive to moving objects	58
Responsive to tactile stimuli	11
Responsive to auditory stimuli	6
Unclassified or unresponsive	330

(Supported by NIMH grants MH 14276, MH 08936, and MH 05507)

NEUROMUSCULAR JUNCTION

1175 ANAEROBIC MAINTENANCE OF SYNAPTIC TRANSMISSION AT THE CRAYFISH NEUROMUSCULAR JUNCTION. Juan Acosta-Urquidi* and H.L. Atwood. Dept. Zool., Univ. Toronto, Toronto, Ont., Canada.

In the presence of 2,4 dinitrophenol (DNP, 0.4-0.8 mM), an uncoupler of oxidative phosphorylation, stimulation of the single excitor motor axon of the leg-opener muscle in the crayfish, Procambarus clarkii, leads to depression of synaptic transmission, indicating depletion of available transmitter stores. However, depression is generally not complete and considerable recovery can occur. When stimuli are delivered at 10-15 Hz, an initial potentiation of EPSP's occurs, followed after 7-14 min. by progressive depression which follows an exponential time course. The time constant of decay is of the order of 12 min., and varies for different synapse types. Most (95%) DNP rundowns level off at a low steady state of release (ca. 0.5 mv), which can continue for many minutes (up to 20-30) if axonal conduction block does not ensue. After a rest period (1 to 15 min.), another cycle of EPSP potentiation and decay can be evoked by further stimulation. Longer rest periods result in greater recovery, and 3 to 5 successive cycles can be achieved.

Maintained low-level transmission and recovery in DNP suggested that anaerobic production of ATP could be involved in the replenishment of releasable transmitter stores. To test this hypothesis, iodoacetate (IAA) at 10^{-4} to 10^{-3}M, was employed in combination with DNP.

Stimulation in IAA alone produced no depression. When IAA was added to DNP during stimulation, complete depression was achieved after one cycle of stimulation, indicating severe depletion of available transmitter stores. Allowing a rest period and then resuming stimulation led to minimal or no recovery. At most, EPSP's interspersed among failures were recorded over the first 1 to 3 min. of resumed stimulation. These results strongly suggest that maintenance of synaptic transmission during repetitive stimulation requires ATP, and that some of the necessary ATP can be supplied by anaerobic metabolism. (Supported by grants from the National Research Council of Canada and the Muscular Dystrophy Association of Canada and CONACYT, Mexico to JAU)

1176 EFFECT OF TETRAETHYLAMMONIUM BROMIDE ON THE ENDPLATE CURRENT OF FROG SARTORIUS MUSCLE. M. Adler, E. X. Albuquerque, M. E. Eldefrawi* and A. T. Eldefrawi.* Dept. Pharmacol. & Exp. Therap., Univ. Maryland, Sch. of Med., Baltimore, MD 21201.

The effect of tetraethylammonium bromide (TEA) was investigated on the endplate region of frog sartorius muscle using a conventional voltage-clamp technique. Endplate currents (EPCs) were recorded from glycerol-treated preparations at 22°C between -180 and +60 mV. Under control conditions, the current-voltage relationship was linear between +60 and -100 mV, and exhibited a small upward curvature with more extreme hyperpolarization. In the presence of TEA ($2x10^{-5}$ to $2x10^{-3}$ M) the current-voltage relationship became markedly nonlinear at hyperpolarized command potentials. With TEA concentrations of $1x10^{-4}$ M or greater, the current-voltage relationship was characterized by an initial linear segment, an intermediate nonlinear segment, and a region of negative conductance. The transition from linearity was dose-dependent; the linear segment extended from +60 to -90 mV at $1x10^{-4}$ M TEA, and from +60 to -40 mV at $1x10^{-3}$ M. Below $1x10^{-4}$ M the current-voltage relationship was still nonlinear, but without a region of negative conductance. The action of TEA was not measurably time-dependent, since similar current-voltage profiles were obtained with command potential durations ranging from 2 sec to 2 msec.

In addition to altering the current-voltage relationship, TEA, in the same concentration range, reduced the dependence of the EPC half-decay time (HDT) on membrane potential. The reduction in the voltage sensitivity of the EPC decay was enhanced with increases in TEA concentration up to $1x10^{-4}$ M, where the HDTs became approximately independent of membrane potential. Higher concentrations of TEA ($2x10^{-4}$ to $2x10^{-3}$ M) reversed the slope of the HDT-membrane potential relationship. The drug-induced alterations in the EPC were found to be independent of the initial holding potential, the interval between steps, and the sequence of potential changes. TEA was without effect on the EPC reversal potential and produced no significant lengthening in the action potential duration even at $2x10^{-3}$ M.

In concomitant biochemical studies, TEA ($4x10^{-4}$ M) inhibited the binding of [^{3}H]perhydrohistrionicotoxin to the acetylcholine receptor enriched microsacs (42% inhibition) and to the solubilized histrionicotoxin binding protein (55% inhibition) from Torpedo electroplax. These results suggest that TEA alters neuromuscular transmission by acting on the ion conductance modulator of the acetylcholine receptor at sites similar to those of the histrionicotoxins. (Supported in part by U.S.P.H.S. Grant NS-12063.)

1177 ACTION OF COLCHICINE, CYTOCHALASIN B, SODIUM THIOPENTAL AND DECANOL ON THE ACETYLCHOLINE RECEPTOR. Roger Anwyl* and Toshio Narahashi. Dept. Physiol. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710 (Present address: Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611).

The action of colchicine, cytochalasin B, sodium thiopental and decanol was examined on the extrajunctional acetylcholine (ACh) receptor of 5-10 day old denervated rat soleus muscles using intracellular recording techniques. The sensitivity of the muscles to iontophoretically applied ACh was 300-500 mV/nC. Perfusion of colchicine, cytochalasin B, thiopental or decanol all reduced the amplitude and rise time of the ACh potential in a manner dependent on the concentration. A 50% reduction in the amplitude of the potential was caused by 2×10^{-4} colchicine, 8×10^{-5} M cytochalasin B and 5×10^{-5} M thiopental. Decanol is insoluble in water, and a 1:10^4 mixture of decanol to saline caused an 80% decrease in the potential amplitude. Increasing the dose of ACh in the presence of colchicine, cytochalasin B, thiopental or decanol caused a large prolongation of the ACh potential, with the half-decay time increasing by up to 20 times, although little or no increase in the rise time occurred. Further increase in the dose of ACh caused the appearance of a second slow peak in the acetylcholine potential. Increasing the concentration of the perfusing drug also increased the prolongation of the decay phase, with decanol causing the greatest, and cytochalasin B the smallest, prolongation. The action of colchicine, cytochalasin B, thiopental and decanol resembles that found previously for local anesthetics. The site of action of these drugs cannot be determined from the present experiments, for although cytochalasin B and colchicine are widely used to disrupt respectively microfilaments and microtubules, they are also known to bind non-specifically to cell membranes, probably by partitioning to membrane lipids. In view of the similarity of action of the structurally diverse drugs used in the present study, we propose that it is most likely that all these drugs are exerting their effect on the ACh receptor by their non-specific interaction with membrane lipids. (Supported by NIH grant 14145).

1178 KINETICS OF CURARE ACTION AT THE FROG NERVE-MUSCLE SYNAPSE. David Armstrong* and Henry A. Lester, Division of Biology, California Inst. of Technology, Pasadena, CA 91125.

We have measured the time course of curare inhibition of the acetylcholine (ACh) response at the postsynaptic membrane of frog sartorious muscle. Curare and ACh were iontophoresed from twin-barreled micropipettes, and the muscle cell's response was recorded intracellularly with conventional techniques. Typical ACh sensitivities were 0.2-0.5 mV/pC. After a pulse of curare, recovery follows a roughly exponential time course independent of the level of inhibition. The rate constant ($1/\tau_{off}$) equals 0.46 ± 0.14 sec^{-1} at -85 mV and 22°C. After a sudden maintained increase in curare release, inhibition is established with an exponential time course whose rate constant ($1/\tau_{on}$) exceeds $1/\tau_{off}$. When the steady-state ACh response is inhibited to 1/n of its control value, $1/\tau_{on} = n(1/\tau_{off})$.

When we reduced the acetylcholine sensitivity at the endplate with cobra toxin, $1/\tau_{off}$ increased. The effect is quite variable, but on the average a ten-fold reduction in ACh sensitivity produces a four-fold increase in $1/\tau_{off}$. Thus the kinetics of curare inhibition depend on the receptor concentration, [R]. This suggests that the time course of inhibition reflects repeated binding of each curare molecule to several receptors as it diffuses within the cleft, rather than the molecular rate constants of the curare-receptor complex. As more receptors are eliminated with toxin, the measured recovery rate should approach the molecular dissociation rate, k_-, of curare from the receptor. We estimate that k_- exceeds 10 sec^{-1}; since curare's $K_D = k_-/k_+ = 4 \times 10^{-7}$ M, the bimolecular association rate constant must exceed 2.5×10^7 $M^{-1}sec^{-1}$. From equation 7 of Colquhoun et al. (1977 J. Physiol. 266, 388), $[R] = K_D(k_-\tau_{off} - 1)$. Then the receptor concentration in the cleft must exceed 10^{-5} M.

1179 PRESYNAPTIC EFFECTS OF ERYTHROSIN B ON NEUROMUSCULAR TRANSMISSION. George J. Augustine, Jr. and Herbert Levitan. Dept. of Zoology, University of Maryland, College Park, Md. 20742.

The anionic dye Erythrosin B (FD&C Red No. 3), a fluorescein derivative, was previously shown to alter the physiological properties of both the pre- and postsynaptic elements of the frog neuromuscular junction (Neurosci. Abstr. 2: 708, 1976). The frequency and mean amplitude of spontaneous miniature endplate potentials (MEPPs) as well as the mean amplitude of evoked endplate potentials were increased by addition of 20-100 micromolar Red 3. The membrane potential of the postsynaptic muscle fibers was also increased by comparable dye concentrations. Using standard electrophysiological techniques we have attempted to determine the mechanism(s) underlying the presynaptic action of Red 3. The frequency of MEPPs increased approximately exponentially with time after application of the dye. Within 5 minutes of perfusion with frog Ringers containing 10^{-4}M Red 3 the frequency increased 2-10 fold and after 30 minutes was 10-100 times greater than before treatment. The effects of brief exposure to moderate concentrations of the dye were reversible only with extensive washing with normal Ringers. The dye-induced increase in MEPP frequency also occurred when the junction was bathed in calcium-free Ringers to which 2 mM EGTA was added. The increase in frequency after dye addition to calcium-free EGTA medium was not significantly altered by increasing the external potassium concentration from 2.5 mM to 12.5 mM. The higher potassium concentration caused significant increases in MEPP frequency in normal Ringers, presumably by causing a depolarization-induced increase in calcium conductance and subsequent influx of calcium down its concentration gradient. Since the frequency of MEPPs increased even in the absence of external calcium we conclude that the dyes presynaptic action is not due to an increased influx of calcium. The fact that the MEPP frequency did not decrease when higher concentrations of potassium were added to calcium-free EGTA medium containing Red 3 indicates that calcium does not flow out of the terminal when calcium conductance is increased by the presence of high potassium. This suggests that the calcium gradient is not adequate for calcium efflux, either because the internal calcium is not significantly increased or extracellular calcium levels are excessive. The action of Red 3 may therefore be due to the release of subtle amounts of calcium stored in the presynaptic terminal or to some other mechanism which promotes synaptic vesicle-presynaptic membrane fusion. (Supported by NSF grant GB-43141)

1180 RELEASE OF ENDOGENOUS ACETYLCHOLINE FROM THE PERFUSED DIAPHRAGM. George G. Bierkamper* and Alan M. Goldberg, Dept. of Environmental Health Sciences, Division of Environmental Toxicology, The Johns Hopkins Univ., Baltimore, MD 21205.

A model for studying the release of endogenous acetylcholine has been developed using the isolated perfused rat hemidiaphragm. The left hemidiaphragm with intact phrenic nerve is dissected from 300 to 400 g rats and placed in 6mM HEPES buffer (pH 7.4, 23°C) containing (in mM):111 NaCl, 2.5 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$ 11 glucose and 0.030 choline bromide. After cannulating the diaphragmatic vein, the preparation is mounted in a closed oxygenated chamber. The muscle is then perfused at the rate of 30-50µl per min. Experiments in which a permeating dye was used revealed that the entire muscle is perfused every 30 sec. at this perfusion rate. Paraoxon, an excellent inhibitor of acetylcholinesterase (AChE), cannot be used in this preparation since early experiments demonstrated that at concentrations sufficient to inhibit AChE it produces rapid degeneration of the muscle. Therefore, in subsequent experiments, Diisopropylfluorosphosphate (DFP, 1.5×10^{-5}M) was added to the perfusion medium for the first 30 min. giving total inhibition of AChE. For the remainder of the experiment physostigmine is maintained in the medium at a concentration of 7×10^{-6}M in order to sustain complete inhibition of AChE. This was verified by direct assay of AChE after homogenization of the muscle and also by complete recovery of radiolabelled ACh after perfusion through the muscle. Following either a condition of rest (spontaneous release) or stimulation (5/sec) of the phrenic nerve, the perfusate is collected and ACh measured by enzymatic radioassay. Preliminary studies indicate that during resting conditions the release of ACh is approximately 1 pmol/min and is increased 6 fold during stimulation. This preparation offers several distinct advantages over other conventional methods. The small volume of perfusion necessary to maintain the preparation facilitates the determination of released endogenous compounds. The ability to perfuse the preparation also allows maximal control for the application and study of xenobiotics. Further, the perfusion system increased the longevity of the preparation so that it is viable for at least 5 to 6 hours. (Supported in part by grants from NIEHS 00454 and 00034.

1181 SOME EFFECTS OF LEAD IONS ON TRANSMITTER RELEASE AT RAT NEUROMUSCULAR JUNCTIONS. Joel C. Bornstein* and Jackson B. Pickett* (SPON: R.A. Fishman). Depts. Physiol. and Neurol., U.C.S.F. San Francisco, CA. 94143.

Little is known about the effects of lead (Pb) on transmission at a mammalian neuromuscular junction (nmj). We have used conventional intracellular recording techniques to examine the effects of Pb on both evoked and spontaneous transmitter release at nmjs in the rat phrenic nerve-diaphragm preparation.

Pb causes a dose dependent reduction in the quantal content of endplate potentials (epps) evoked in a low calcium (Ca)/high magnesium bathing solution by 0.5 Hz suprathreshold stimulation. For example, 0.1mM Pb produces a total block of evoked release, while 5 micro-molar Pb reduces the quantal content by 30%-50%. This effect of Pb is reversed by increasing the extracellular concentrations of Ca ([Ca]) which suggests that the mechanism of action of Pb is similar to that of other divalent cations in antagonising the entry of Ca into the nerve terminal. This hypothesis is supported by the finding that 0.1mM Pb caused a marked shift to the right of the curve relating quantal content to [Ca].

In contrast to the effects of Pb on evoked release, the frequency of spontaneous release is accelerated by concentrations of Pb in excess of 0.05mM although this increase is considerably less than that produced by similar Pb concentrations at frog nmjs (Nature 243:354, 1973). Lead is known to inhibit the uptake of Ca into mitochondria (Brain Res., submitted) and it seems likely that this effect of Pb on spontaneous release is due to an increase in the cytoplasmic concentration of Ca similar to that produced by other inhibitors of mitochondrial Ca uptake (J. Physiol. 248:285, 1975).

Thus, Pb at concentrations equivalent to those seen clinically has significant effects on the properties of transmitter release at mammalian nmjs. However, it remains to be seen whether these acute effects can account for all the symptoms seen in chronic lead poisoning.

1182 CONTRACTILE PROPERTIES OF FAST AND SLOW MUSCLE FOLLOWING GLUCOCORTICOID-INDUCED MYOPATHY. B.R. Botterman*, P.F. Gardiner*, E. Eldred, and V.R. Edgerton. Depts. of Anatomy and Kinesiology, and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

A striking feature found in steroid-induced myopathy is the selective involvement and atrophy of fast-twitch fibers. Since this fiber selectivity might lead to altered contractile properties, histochemical observations and the assessment of several contractile parameters in cat soleus (S) and medial gastrocnemius (MG) muscles were made following two weeks of daily injection (IM) of triamcinolone (4 mg/kg).

Mean muscle weights for S and MG were reduced by 18% and 37% respectively, when compared to control muscles. However, comparisons of muscle/body weight ratios revealed that the S ratio was unchanged, while that of the MG was decreased due to the treatment. In the MG, fast-twitch glycolytic fibers were reduced in cross sectional area to a greater degree (51%) than fast-twitch-oxidative glycolytic fibers (35%), with slow-twitch-oxidative (SO) fibers the least affected (33%). SO fibers in the soleus were reduced in area by 11%. The MG and S of the treated animals showed smaller absolute maximum twitch (P_t) and tetanic (P_o) tensions; however, Po, when expressed per gram of muscle or contractile protein, was not different between the groups for either S or MG.

Speed-related contractile parameters of S and MG were influenced by steroid treatment. Soleus muscles of treated cats had shorter contraction times (72.9 ±2.9 vs. 85.7 ±2.6 ms) and half-relaxation times (73.7 ±3.5 vs. 103.5 ±5.0 ms). However, MG showed longer contraction times(33.8 ±0.9 vs.29.9 ±0.6 ms) and half-relaxation times (26.6 ±1.0 vs. 22.6 ±0.5ms) in the steroid group. No change in either S or MG was seen for the maximum rate of tension development (%P_o/ms). Fusion of S occurred at higher stimulation frequencies while MG fused at lower frequencies compared to control muscles.

As shown by Riker et al. (Arch. Neurol. 32:688, 1975), S post-tetanic potentiation (PTP), which is primarily neurogenic in origin, was enhanced, but might be explained partly by decreased normalized P_t and twitch-tetanus ratios. Conversely, PTP in the MG, which is primarily myogenic, was decreased. Our findings suggest that steroid treatment has direct effects on muscular components of fast- and slow-twitch muscle fibers, as well as influencing neuronal excitability. In addition, the muscle specificity is not solely a function of fiber composition, since SO fibers in S and MG were not equally affected.

1183 NEUROMUSCULAR TRANSMISSION IN HUMAN CONTROL AND PATHOLOGICAL SINGLE MOTOR UNITS. William F. Brown, Hytham A. Kadrie* University Hospital, London, Ont., Canada

In humans, neuromuscular transmission is tested in clinics by measurement of the changes in the maximum compound potential or tensions evoked by presynaptic trains of supramaximal stimuli or, more recently, by measurement of the 'jitter' at individual junctions using the single fiber EMG electrode. Neither method, however, makes it possible to learn if motor units (MU) in human neuromuscular transmission disorders are attacked at random; or if not, the type and proportion of MU most abnormal.

The questions have been investigated by testing single MU by trains of presynaptic stimuli (frequencies 0.1 to 10 per second), ischemia and tetanic stimulation. Because there are established relations of MU surface voltage to tension and conduction velocity, MU low to high in peak-to-peak (p-p)voltage were tested. Control human MU have significants in the p-p voltage linked to reductions in the p-p duration, maximum at 10 per second but present at 1.0 per second; no significant differences in the low-medium or high surface voltage being observed. In neuromuscular diseases, decrements in the p-p voltage of single MU much more striking than in the corresponding maximum compound potentials were observed at stimulus frequencies of 3 and 10 per second. Decrements were clearly most pathological in the low surface voltage MU, units that had the longest latencies (adjusted to a standard distance). The predilection of SMALL MU occurred in myasthenia gravis (proportion of abnormal MU varied from 0 to 90% from patient to patient), motor neurone disease and peripheral nerve diseases having evidence of abnormal neuromuscular transmission.

Though the findings could suggest a neurogenic factor in myasthenia gravis, the universal selection of SMALL MU in all diseases tested to date could be interpreted by a hypothesis that neuromuscular failure occurs first in SMALL MU because even in health the margin for safe neuromuscular transmission is less in SMALL than larger MU, the result being higher risk of synaptic failure in SMALL MU even in diseases that attack MU and junctions at random.

1184 ACUTE EFFECTS OF ACETALDEHYDE ON MOUSE NEUROMUSCULAR JUNCTION- AN ELECTROPHYSIOLOGICAL STUDY. Peter L. Carlen, William A. Corrigall, and Allan L. Staiman. Neurobiology Lab., Dept. of Medicine (Neurology), Addiction Research Foundation, Toronto, Ontario, Canada. M5S 2S1.

Acetaldehyde, the first metabolite of ethanol, has been implicated in acute and chronic effects of alcohol. Effects of acute acetaldehyde were examined in vitro on the phrenic nerve-hemidiaphragm neuromuscular junction of the mouse at 20°C in a superfusion bath. The end-plate potential (EPP) evoked at 1/second stimulation was blocked at acetaldehyde concentrations between 3 - 25mM, and the quantal content (as measured by the failure method) paralleled the reduction in EPP height. During development of the EPP block, the EPP latency was increased. Acetaldehyde concentrations between 3 - 25mM were found to block the compound action potential (CAP) and the nerve terminal spike. Average times for complete reduction of the CAP and the EPP were 29.9 min. and 18.5 min. respectively. Miniature endplate potential (mepp) height and shape were unaltered at these concentrations, suggesting a purely pre-synaptic action. MEPP frequency was observed to 1) decrease only at lower concentrations, 2) decrease initially and then increase at higher concentrations or 3) increase only at high concentrations. The effect of acetaldehyde on mepps is therefore distinct from that of ethanol (in which case there is a monotonic increase in mepp amplitude and frequency with ethanol concentration. Average time of onset of mepp frequency reduction was 3.5 min., if it occurred. Average time for a mepp frequency increase was 14.4 minutes. All effects reported were completely reversible on removal of acetaldehyde. Bath-acetaldehyde concentrations were assayed using gas-liquid chromatography.

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1185 PROLONGED FACILITATION OF TRANSMITTER RELEASE IN NEUROMUSCULAR JUNCTIONS OF CRAB STOMACH MUSCLES. Milton P. Charlton*, C. K. Govind and H. L. Atwood (SPON: K. Livingston). Zoology Dept., University of Toronto, Toronto, Canada, M5S 1A1.

Facilitation of transmitter release was studied in neuromuscular junctions of the intrinsic stomach muscles (Gm8b) of the Blue Crab (Callinectes sapidus). Seven seconds following a single motor nerve impulse, transmitter released by a second stimulus was increased by as much as 40%. The maximum facilitation immediately following a stimulus was 300%. This facilitation decayed from its initial value along two exponential time courses with time constants of about 100 msec for the intial fast component and 2-5 sec for the secondary slow component. The decay of facilitation was unusually prolonged in these synapses in comparison with synapses in amphibians, squid and arthropods. Moreover, the time constant of decay of the slower component does not appear to be affected by changes in temperature as is the decay of facilitation in squid and toad synapses. The results support the idea that there are several different mechanisms which cause facilitation and its decay.

1186 INFLUENCE OF SUCCINIC ANHYDRIDE ON THE DECAY OF ENDPLATE CURRENTS EVOKED BY HIGH FREQUENCY STIMULATION. Jose del Castillo and Gladys Escalona de Motta*. Lab. of Neurobiol., Sch. Med., UPR, San Juan, P.R. 00901

Saji & del Castillo (J. Neurosc. Res. 1:437, 1975) observed that small organic molecules containing either the ester (-O-CO-) or the carboxylic anhydride (-CO-O-CO-) groups depolarize the endplate membrane of frog muscle. In addition, electro-osmotically applied doses of these compounds, which produce a depolarization of only a few mV, exert a marked potentiating action on the amplitude of iontophoretic acetylcholine (ACh) potentials. Similarly, a potentiation of the effects of bath-applied ACh was observed when these compounds were added to the external solution in concentrations of up to 1 mM. One of them, succinic anhydride (SA) was selected to study further the mechanism of action of this class of compounds.

While confirming all the above effects, we were surprised to find that SA (1 mM) has no significant influence on the average amplitude of the miniature endplate potentials and increases by a factor of only 1.1X the amplitude of the endplate potentials. This is in marked contrast with its potentiating action on the depolarizing effect of bath-applied ACh, (10 uM ACh; 1 mM SA) which increases, on the average, by a factor of 1.5X (range 1.2X to 2X). The conclusion was reached that SA does not act on the ACh-receptors but rather that it accelerates the inward movement of ACh by saturating binding groups located within the diffusional pathways through which ACh must pass on its way from the bath solution to the receptors.

To see whether SA would accelerate also the diffusion of ACh in the opposite direction (i.e. from the synaptic cleft to the bath) we have recorded the decay of the endplate currents (epcs) in frog muscles treated with neostigmine (3 uM). We used sciatic-sartorius preparations electromechanically uncoupled by pretreatment with 1.5 to 2.0 M formamide-Ringer's. From 1 to 4 stimuli, separated by 5 msec intervals, were applied to the nerve while recording the epcs with a two microelectrode voltage clamp system which held the transmembrane potential at -100 mV.

While the half-decay time of the first and second epcs are not changed by SA (1 mM), this compound significantly shortens the half-decay times of the 3rd and 4th epcs. This observation supports the conclusion that SA acts by decreasing the efficacy of a diffusional barrier located away from the ACh-receptors.

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1187 TEMPERATURE INDUCED ALTERATION OF MUSCLE TWITCH RESPONSE AND CORRELATED ULTRASTRUCTURAL CHANGES OF MOTOR ENDPLATES IN IN VITRO STUDIES. Anna B. Drakontides. Department of Anatomy, New York Medical College, Valhalla, NY 10595.

In vitro preparations are routinely employed for the study of many biological phenomena. Mammalian nerve-muscle preparations are maintained at temperatures ranging from 23-37C. Many investigators, however, have shown that the ambient temperature has a profound effect on the spontaneous release from motor nerve terminals in the frog, and in the frequency of miniature endplate potentials at mammalian neuromuscular junctions. The present in vitro studies show that ambient temperatures of 37C for periods in excess of 4 hours result in the "decay" of the twitch response and that correlated degeneration-like changes are evident at motor nerve terminals. The indirectly evoked isometric muscle contractile response to single supramaximal rectangular pulses of .05 ms duration, at .05 Hz was recorded from phrenic nerve hemidiaphragm preparations of albino rats (150-300 gms). After 4 hours at 37C muscle responses were reduced by 30% and by 8-10 hours by 85-90%. The twitch tension was still within 47% of initial control values after 10 hours in preparations maintained at 30C, while at 23C responses were 90% of initial control following 10-12 hours. A 50% reduction in twitch tension occured after 28 hours at 23C. An electron microscopic study of motor nerve terminals from in vitro diaphragm preparations maintained at 37C revealed structural alterations which were directly related to the degree of twitch tension loss. These changes included a reduction in numbers of vesicles, vesicular clumping, the appearance of membrane-bound clusters of vesicles, an increase in membrane profiles within terminals, mitochondrial swelling, and the presence of numerous vesicular profiles between the secondary junctional folds. All the motor nerve terminals samples from in vitro preparations at 23C sustained for periods as long as 20 hours were normal in structure. Continuous stimulation at .05 Hz was not the cause of changes seen in function and structure at 37C, since preparations maintained in vitro and intermittently monitored exhibited the same changes in twitch loss and morphological alterations. The changes seen at 37C probably reflect increased metabolic activity and energy requirements. In the evaluation of in vitro experiments, therefore, consideration should be given to the alterations that may be induced by the factor of the ambient temperature.

1188 STUDY ON THE MODE OF ACTION OF BLACK WIDOW SPIDER (BWSV) ON VERTEBRATE NEUROMUSCULAR JUNCTION. Alfredo Gorio*, Lee L. Rubin* and Alexander Mauro*. (SPON: F. Brink, Jr.). Rockefeller Univ., New York, NY 10021

Application of BWSV at vertebrate neuromuscular junctions produces, even in the absence of Ca^{++}, a transient increase in mepp frequency and leads eventually to a neuromuscular block and depletion of synaptic vesicles. Since artificial lipid membranes treated with B_5 (the fraction which is active on vertebrate neuromuscular junction) show a great increase in cation conductance, it has been postulated that the entrance into the nerve terminal of Na^+, in the Ca^{++} free medium, releases internal stores of Ca^{++}, thereby secreting neurotransmitter. Since glucosamine is impermeant both to the venom-treated artificial lipid membranes and the postsynaptic membrane of frog muscle, we assumed that it is also impermeant at the nerve terminal. Thus we used glucosamine as a Na^+substitute to test the hypothesis of the ionophore action of B_5. During perfusion in glucosamine Ringer's, of course, no electrical activity can be recorded. The recovery in Ringer's of the response is dependent on the perfusion time and the position of the muscle fiber. In surface fibers, after 30' perfusion in glucosamine Ringer's, mepps reappear in 30"-60" and epps a few seconds later. After 1 hour perfusion, the recovery time is of the order of 3-4 minutes. Ultrastructural analysis of frog cutaneous pectoris muscle fixed after either 30' or 60' in glucosamine Ringer's shows that nerve terminals retained their normal structure. When BWSV is applied in 0 Ca^{++}, glucosamine-Ringer's a good depletion of synaptic vesicles is already noticeable after 15'. After 60' the nerve terminals are depleted of vesicles and the mitochondria appear unchanged in number and structure. On the other hand when Ca^{++} is added to the Na^+ free Ringer's the mitochondria are very swollen. After 15' action of BWSV in glucosamine Ringer's no epps can be recorded and the mepps frequency resembles that at the end of the venom discharge; after 60' treatment only rare mepps can be observed. When muscles are fixed after 1 hour treatment with large doses of BWSV in 0.5 mM Ca^{++}, 4 mM Mg^{++} Ringer's, the nerve endings are greatly swollen and devoid of mitochondria. Most likely swelling and disaggregation of mitochondria are due to an enormous Na^+ entry. From these results we can conclude that BWSV can act in two ways at the nerve terminal: one is to open channels permeable to cations such as Na^+ and Ca^{++} and the other is to stimulate release by a mechanism, yet to be established, which may not involve the ionophore action.

1189 THE ACTION OF CHLORPROMAZINE AND PHENYTOIN ON MUSCLE RIGIDITY DUE TO CEREBELLAR LESIONS. Verne D. Hulce. Department of Pharmacology, Georgetown University, Washington, D.C. 20007

The potentiation of the action of chlorpromazine by phenytoin in reducing the extensor rigidity of decerebration was reported by Anderson and Raines (Neurol. 26:858,1976). Lesions to the anterior cerebellar cortex in cats also produce an extensor rigidity. This rigidity is evoked as a short acting phenomenon after stimulation and lasts up to two-minutes after each stimulation. Lesions were produced in the cerebellum by the aspiration of Larsell's lobules II to V by suction. To test, vestibular stimulation was produced by rotating the whole cat about the rostral-caudal axis or by rapidly lowering the animal. The time course and forces of extensor rigidity were determined from the peak force required to collapse the extended limb measured against a force transducer and recorded on a polygraph. Electromyograms were obtained simultaneously to assist in timing the duration of the evoked extensor rigidity. Chlorpromazine was given as a solution in saline by i.m. injection and phenytoin was administered via the i.p. route suspended in a 0.5% methyl cellulose solution. Both drugs were tested various times after dosing to find a peak effect. The effects of phenytoin and chlorpromazine were found to be distinctly different suggesting different modes of action. Chlorpromazine reduces, in a dose dependent manner, the peak force after vestibular stimulation. Phenytoin, in low doses, reduces the total time of extensor rigidity seen after vestibular stimulation but has no effect on peak force development. As specific examples of these effects, a dose of 40 mg./kg. of phenytoin reduced the time of extensor rigidity from one minute and 56 seconds to less than 5 seconds with no appreciable effect on the peak force. On the other hand, chlorpromazine at a dose of 2 mg./kg. had no effect on the duration of rigidity but reduced the peak force from 3.7 kg. to 1.0 kg. Preliminary studies on the pharmacological interaction of these drugs suggests that their effects are additive but over the two dimensions of force and time. The ability of one drug to potentiate the other in reducing decerebrate rigidity is not seen in the decerebellar rigidity model unless a force-duration product is considered as the dependent variable.
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1190 CHARACTERISTICS OF NEUROMUSCULAR JUNCTION AFTER THE COMPLETE RECOVERY FROM DEPRESSION BY SUCCINYLCHOLINE. K. C. Kim. Dept. Anesth. Sch. Med., Indiana Univ., Indpls. Ind. 46202.

This study is to investigate the nature of neuromuscular junction (N.M.J.) after the muscle twitch height completely returned to the control height from the depression by succinylcholine (Scl). METHOD: Twelve mongrel dogs were anesthetized with pentobarbital 35 mg/kg i.v. Achilles tendon was severed and attached to the force transducer for continuous recording of the muscle twitch. The nerve to the gastrocnemis muscle was stimulated with a supramaximum stimulus every two seconds. Scl, edrophonium (Edp) and neostigmine (Nst) were administered in a 0.3 ml volume of saline solution. They were injected into an artery supplied to the gastrocnemis muscle. Edp was given at various intervals after the administration of Scl. RESULTS: When 0.2 mg Scl i.a. was given, the mean total time from the depression to the complete recovery of muscle twitch by Scl was 16 min $\pm$ 6. The muscle twitch was depressed when Edp was given. The depressing effect of Edp lasted for 19 min $\pm$ 11 after the muscle twitch had completely recovered from the Scl depression. The depressing effect of the Edp gradually shifted to the stage where muscle twitch had no response by Edp, then to the muscle twitch augmentation, which lasted for 37 $\pm$ 13 minutes. The augmentation period was determined by the time required to reach the control augmentation height of Edp from the muscle twitch reach to the control height with no response by Edp. The experiment was repeated with neostigmine 0.5 mg given i.a. 10 minutes before Scl administration; the depression, no response, as well as augmenting effect of Edp previously described was significantly prolonged than that of Scl (122% and 178% respectively). The mean total time for recovery of the control state from the neuromuscular blockade with neostigmine 0.5 mg and 0.2 mg Scl was 179 min., which was 2.7 times prolonged by neostigmine than that of Scl.

SUMMARY: This data suggests that this sequence of the characteristic changing of the N.M.J by Edp (depression, no response, and augmentation) after Scl or Nst with Scl could explain the inconsistent response by anticholinesterase to the dual blocks. A test dose of Edp can aid in ascertaining the status of the muscle. This will arrow a method of determining the usefulness of Edp in reversing the relaxation resulting from Scl.

1191 FORMATION OF THE MYOTOMAL NEUROMUSCULAR JUNCTION IN XENOPUS LAEVIS DURING NORMAL DEVELOPMENT: AN ELECTROPHYSIOLOGICAL AND FINE STRUCTURAL STUDY. R.W. Kullberg*, T.L. Lentz and M.W. Cohen. Dept. Physiol., McGill Univ., Montreal, P.Q. and Sect. Cytol., Sch. Med., Yale Univ., New Haven, Conn.

Spontaneous potentials, considered to be MEPPs, were detected by intracellular recording as early as stage 21, and by stage 24 they were observed in every embryo tested. Like MEPPs at later stages they were blocked by curare but not by tetrodotoxin. EPPs, subject to block by tetrodotoxin, were evoked by electrical stimulation of the spinal cord in embryos as young as stage 24 and occurred spontaneously as early as stage 22. The durations of MEPPs and EPPs were initially relatively long. Focal external recordings revealed an eight-fold decrease in duration during the course of development.

Nerve processes emerged from the spinal cord and contacted developing muscle cells as early as stage 21 but junctional specializations were not apparent and vesicles were rare even in stage 24 embryos. During the next 24 hr, between stages 25-36, vesicles increased in number and became localized towards the junctional surface of the nerve ending. Basement lamina developed in the cleft and postjunctional ridges and densities were observed. Individual muscle cells also became contacted by several nerve processes. By stages 48-52 there were fewer contacts on individual muscle cells, and Schwann cell processes partially covered the nerve endings. Gap junctions were observed between muscle cells throughout development but occurred less frequently at the later stages.

It is concluded that by the time they reach the muscle cells, or very shortly thereafter, at least some of the growing nerve processes can release transmitter and some of the muscle cells are sufficiently sensitive to acetylcholine in the region of contact to respond with millivolt depolarizations. These earliest functional contacts are however morphologically undifferentiated.

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1192 EVIDENCE THAT β-BUNGAROTOXIN ARRESTS SYNAPTIC VESICLE RECYCLING BY BLOCKING COATED VESICLE FORMATION. N.L. Lassignal* and J.E. Heuser* (SPON: B. Libet). Dept. Physiol., Univ. of Calif., San Francisco, Calif. 94143.

While documenting the phospholipase action of β-bungarotoxin (β-BTX) on the frog neuromuscular junction, we observed that "coated pits" became abundant in some of the nerve terminals that we fixed at about the time transmission was blocked by the toxin (Neurosci. Abst. II: 718, 1976). Later, we noted that coated pits were also abundant in many of Tsai's electron micrographs of β-BTX treated rat diaphragms, especially the ones he obtained from rats that were dying from β-BTX injections (M-C. Tsai, Ph.D. Thesis, Taiwan Univ., 1975). We wondered if this meant that β-BTX enhances this sort of endocytosis, as one of its effects; or whether, on the contrary, β-BTX blocks the formation of coated vesicles and thus produces a "traffic jam" of coated pits.

One indication that the toxin might *block* the coated vesicle formation necessary for recycling of synaptic vesicles was an apparent contradiction in the early β-BTX literature: Chen and Lee first reported that synaptic vesicles had *disappeared* from the diaphragms they removed from rats that were dying from β-BTX injections (Virch. Arch. B Zellpath. 6: 318, 1970). But then they reported that synaptic vesicles were *normally abundant* in diaphragms they bathed in the toxin in vitro and did not stimulate (J. Pharm. Exp. Therap. 184: 339, 1973). Thesleff et al (Neurosci. 1: 175, 1976) observed the same difference while studying the effects of stimulation on the neuromuscular block produced by two other β-toxins closely related to β-BTX, and suggested that these two toxins might exaggerate vesicle disappearance during stimulation by blocking synaptic vesicle reformation.

We here report the results of stimulating frog cutaneous pectoris muscles one hour after they were placed in 2 μg/ml β-BTX in normal Ringer at 22°C. (1) Freeze-fractures of these muscles showed that stimulation led to a great increase in the proportion of nerve terminals that were covered with shallow coated pits -- that is, covered everywhere except at the active zones where exocytosis occurs. (2) Thin sections of these muscles showed that the plasmalemmal pits produced during stimulation in β-BTX were coated with a "basketwork" structurally similar to the "clathrin" that surrounds coated vesicles. (3) Histochemistry of HRP as a marker for endocytosis showed that in spite of all these coated pits, stimulation *did not* produce the usual uptake of HRP into coated vesicles and synaptic vesicles that it does in normal nerves. Such a total block of HRP uptake in β-BTX indicates that the proliferation of coated pits that we see represents *a block of endocytosis*. This further strengthens our idea that coated vesicle formation is the first step in synaptic vesicle recycling.

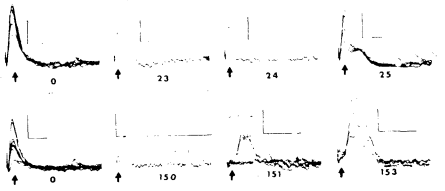
(Supported by the Muscular Dystrophy Association of America.)

1193 PRESYNAPTIC EFFECTS OF CENTRUROIDES SCULPTURATUS VENOM(CSV) ON FROG NEUROMUSCULAR JUNCTIONS (NMJ). H.E. Longenecker, Jr., J. Bruck* and G.L. Longenecker, Depts. of Physiol./Pharmacol., U. of So. AL, Mobile, AL 36688

Previous investigators demonstrated that CSV contains neurotoxins that alter Na conductance of neurons causing repetitive firing. There are reports on curarized NMJ preparations of CSV induced repetitive firing; however, it was impossible to observe miniature (M) endplate potentials (EPP) or to rule out any post-synpatic venom effects. Thus, we report results from NMJs in which twitch was blocked by reduction of Ca to .5mM with addition of 4mM magnesium (pH =7.4).

In 16 preparations CSV was added to NMJ bathing solution (0.56 to 4.48ug/ml). Repetitive firing of the nerve(NT) was evidenced by post-stimulus repetitive bursts of EPPs. This phenomenon developed gradually and then disappeared. The exact sequence was highly variable (max number of repetitions: 5-50; peak time of max: 5-100 minutes). Occasionally, unstimulated bursts of EPPs were observed.

In several experiments prolonged EPPs were observed (23,25,151,153 min). Since potentials with similar time course have been observed in neurons, we assume that since the nerve would be depolarized for a longer period of time, the release probability is similarly prolonged, thus the elongated EPP. The jagged EPP (t=151,153 min) is the result of superposition of MEPPs on the normal EPP elicited by the prolonged NT potential.



MEPPs were recorded in all preparations. No consistent change in MEPP amplitude was seen. MEPP frequency(F) remained almost unchanged in 4 experiments. However, in 12 experiments MEPPF rose to values of up to 500 per second with subsequent exhaustion of quantum stores. In another set of 8 experiments with Ca free solutions and no indirect neural stimulation, MEPPF rose in all 8 experiments to high values with subsequent depletion. These effects were abruptly blocked by addition of tetrodotoxin (TTX) and never occurred if TTX was added before CSV. Since it has been shown (Longenecker, Neurosci. Abs. 1976) that neural stimulation in Ca free solution leads to high MEPPF, we conclude that CSV induces high frequency repetitive firing of the nerve in Ca free media, and due to Na entry into the NT intracellular Ca is freed and subsequent transmitter release occurs. (NIH #ES01321-01)

1194 CHRONIC STIMULATION OF FROG NEUROMUSCULAR JUNCTIONS IN VITRO. Katy Lynch* (SPON: T. S. Reese). Geo. Wash. Univ. Med. Sch. Washington, DC 20037 and LNNS, NINCDS, NIH, Bethesda, MD 20014.

Matched pairs of sartorius muscles from R. pipiens, with their attached nerves, were maintained in vitro at room temperature for up to 60 hrs. The experimental apparatus permitted each nerve, ligated and cut at the level of the sacral plexus, to be stimulated and the muscle response recorded with a force displacement transducer. Stimulation of the nerves at 120 times/min caused muscle twitches for over 40 hrs. Twitch tension reached a maximal value within 90 sec, then rapidly declined for 15 min to a plateau level 5.4±1.7% of the maximum. In those muscles stimulated continuously for 24 hrs, twitch tension continued to decline very gradually to 0.9±0.7% of the maximal value. Control muscles, rested 24 hrs in vitro and then stimulated at 2Hz for 20 min, developed maximal tensions 5 to 50% less than those achieved in their fresh state. By 6 min, however, their tension recordings were identical to those of fresh muscles. Direct stimulation of curarized muscles yielded a similar though much more regular pattern of response, with equal or greater declines in twitch strength over 24 hrs. This finding, coupled with intracellular recordings, suggests the following: The overall decrement in the twitch tension of indirectly stimulated muscles is primarily due to fatigue of the muscle fibers rather than failure of neurotransmission. The quantal content of the epps, known to decline rapidly at the onset of repeated stimulation (JCB 54:30, 1972), probably hovers near the level required to trigger action potentials. Fluctuations in the number of contracting fibers throughout the long plateau period would explain the unevenness and persistence of the twitch response. Preliminary morphometric analysis of 40 nerve terminal profiles from muscles rested 24 hrs and stimulated 20 min at 2Hz, and of 40 profiles from muscles stimulated at 2Hz for 24 hrs revealed no significant differences in the number of synaptic vesicles, in plasmalemmal area, or in the concentration or size of mitochondria. We conclude that frog motor endplates can utilize local recycling mechanisms or axonal resources to sustain neurotransmitter release for more than 40 hrs. Available axonal resources were reduced by ligating the sartorius nerve at its branch point from the sciatic or by bathing the sciatic in 20mM colchicine or 0.1mM vinblastine. This produced no change in the twitch pattern over 24 hrs, indicating that rapidly transported materials play a negligible role in sustaining synaptic function over this period.

1195 β-BUNGAROTOXIN: RETENTION OF THE PRESYNAPTIC BLOCKING EFFECT FOLLOWING INACTIVATION OF THE PHOSPHOLIPASE. Leona M. Masukawa, Gene S. Tobias*, Mildred A. Donlon* and David R. Livengood. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD. 20014.

β-bungarotoxin (β-BuTX) has been shown to be a specific presynaptic blocking agent at the neuromuscular junction by a number of investigators. Its mode of action has been postulated to be due ultimately to the disruption of transmitter release mechanisms by its phospholipase activity. The phospholipase activity can be removed by boiling the toxin at pH 8.6. After this treatment the toxin (phospholipase-inactive β-BuTX) was still able to block transmission in in vitro neuromuscular preparations of the frog and rat. Blockade of evoked release of acetylcholine was examined by averaging endplate potentials which previously had been partially blocked with 3μg/ml d-tubocurarine. The blockade due to the phospholipase-inactive β-BuTX was complete and irreversible within 20 minutes at high toxin concentrations (.1 to 1 μg/ml) and was not preceded by a transient increase in evoked release as has been shown for the enzymatically active toxin. The degree of blockade was found to be a function of the toxin concentration. The effect was rapid in onset and reached a steady state level. This action is inconsistent with the action of a phospholipase but is characteristic of a direct binding of the toxin to sites important to transmitter release. Miniature endplate potential (MEPP) frequency was measured in the presence of phospholipase-inactive β-BuTX to study further its mode of action. MEPP frequency appeared to increase in the presence of the toxin, and the distribution of miniature amplitudes was shifted to a population of lower amplitude. This decrease in amplitude may be due to a postsynaptic effect; therefore, acetylcholine sensitivity of extrajunctional receptors of denervated muscle was measured in the presence of phospholipase-inactive β-BuTX. We hypothesize that the fully active toxin contains two active sites: one site specific to the presynaptic terminal which can block release of transmitter and another site which has a phospholipase action.

1196 ULTRASTRUCTURAL ANALYSIS OF FUNCTIONAL MOTOR ENDPLATES FORMED IN SCHWANN-CELL-FREE CULTURES OF MOUSE SKELETAL MUSCLE INNERVATED BY VENTRAL CORD EXPLANTS. Edmund B. Masurovsky, Edith R. Peterson*, George D. Pappas and Stanley M. Crain. Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461

Cultures of fetal mouse ventral spinal cord explants and adult skeletal muscle "regenerates", prepared free of Schwann cells, have been maintained for over 3 months in vitro with stable cross-striations, vigorous contractile activity, cholinesterase-positive neuromuscular junctional foci, and other features of mature innervated muscle fibers (Peterson and Crain, Soc. Neurosci.'76, Abstr. p. 1018). Cultures selected for electron microscopy (EM) were first tested electrophysiologically (see below) immediately prior to fixation either in: a) 2.5% glutaraldehyde in Millonigs' buffer; or b) 1% paraformaldehyde, 1% acrolein, 2.5% glutaraldehyde, 2.5% DMSO and 0.005% $CaCl_2$ in cacodylate buffer (pH 7.3) for 1 hr at 4°C., followed by overnight immersion in 2% paraformaldehyde, 5% glutaraldehyde and 0.005% $CaCl_2$. After post-fixation with 1% OsO_4 (50 min at 4°C), standard dehydration, and Epon-embedment, thin sections were cut and stained with 50% ethanolic uranyl acetate and lead citrate for examination in a Philips 300 electron microscope.

By 5 weeks in vitro, EM revealed bare and occasionally partially invested presynaptic nerve terminals situated within postsynaptic endplate formations with prominent primary and secondary infoldings. While most terminals contained agranular ($\sim$ 50 nm) vesicles, some also contained various dense-core ($\gtrsim$ 80 nm) vesicles. Those nerve terminals partially covered by cells with dense-appearing cytoplasm showed no basal lamina typically associated with Schwann cells, although abundant basal lamina was clearly demonstrated along sarcolemma and within clefts. Furthermore, these dense investing cells containing ribosomes and microtubules sometimes projected $\sim$ 11-12 nm period myelin-like loops next to neurites, suggestive of oligodendroglia (Dr. C.S. Raine, pers. comm.) which had migrated from the ventral cord explant with the outgrowing motor nerve fibers. Subsarcolemmal nuclei, typical mitochondria, ordered myofilament lattices, well-developed sarcoplasmic reticulum and triads denoted overall muscle maturity. Prior to EM, vigorous muscle contractions could be repeatedly evoked by electric stimuli to the cord explants and the contractions were selectively blocked by d-tubocurarine (1 μg/ml), as in cultures with organotypic cord-DRG explants.

These correlative data demonstrate that functional motor endplates can develop in longterm co-cultures of mammalian spinal cord and skeletal muscle in the absence of Schwann cells.

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1197 HYPERPOLARIZING MINIATURE SYNAPTIC POTENTIALS IN THE MOUSE DIAPHRAGM. Owen B. McManus* and James R. Musick. Dept. of Physiol. U. of Utah, Sch. of Med., Salt Lake City, UT. 84132.

Intracellular recordings were made from muscle fibers of the mouse diaphragm by standard microelectrodes. The high input impedance of these fibers allows recording of miniature endplate potentials (m.e.p.p.s) of ca. 2 mV without using anticholinesterases. M.e.p.p. amplitude frequency distributions are usually normal; occasional cells show multimodal distributions with a less frequent subclass smaller than the mean. Some records show hyperpolarizing miniature synaptic potentials (h.m.s.p.s) as well as m.e.p.p.s. H.m.s.p.s have the following properties: 1. A localized intracellular distribution at the motor endplate since they are recorded when external m.e.p.p.s are seen prior to intracellular penetration. 2. Monophasicity. 3. Rapid rise time with slower exponential decay and shorter total duration than m.e.p.p.s due to rise and decay time reduction. 4. Mean peak amplitude similar to simultaneously recorded m.e.p.p.s. 5. Non-normal amplitude frequency distribution due to positive skewness. 6. Lower average frequency than m.e.p.p.s (ca. 30% reduction). However, some cells show frequency increases during bursts and a greater frequency of h.m.s.p.s than m.e.p.p.s. It is suggested that h.m.s.p.s reflect inward positive ionic current recorded in close proximity to receptor-ionophore complexes activated by quantal acetylcholine release, as predicted by Del Castillo and Katz (1956,J. Physiol. 132: 630).

Supported by PHS grant #5 P01 NS07938.

1198 RELEASE OF LOW MOLECULAR WEIGHT AMINO-COMPOUNDS BY NERVE STIMULATION OF PARALYZED NEUROMUSCULAR PREPARATIONS. James R. Musick. Dept. Physiol., Sch. Med., University of Utah, Salt Lake City, UT 84132.

Nerve stimulation of the R. pipiens sciatic nerve-sartorius preparation, paralyzed by curare, increases the efflux of ninhydrin positive material into the bathing saline solution; average release (± S.E.) was 160 ± 45 pmoles leucine equivalents/mg tissue, n = 13. This response has characteristics similar to the release of Lowry-reactive material (Musick and Hubbard, Nature 327:279, 1972). Simultaneous measurement of ninhydrin and Lowry reactivity of tissue effluents allowed determination of the ninhydrin/Lowry ratio (N/L), in the units moles leucine equivalents/mole BSA equivalent. Under control conditions, the N/L ratio was 282 ± 66 and during stimulation at 5/sec, 1939 ± 409 (mean ± S.E., n = 9, $p < 0.01$ by paired t-Test analysis). These results suggest that nerve stimulation releases amino-compounds with minimal Lowry reactivity. Comparison with N/L ratios of purified test compounds indicate that the released substances may be predominately amino acids or peptides and proteins are a minor component. Preliminary molecular size measurements indicate that the released amino-compounds are $\leq$10,000 molecular weight. These results are consistent with the hypothesis that proteolytic enzymes are secreted together with ACh from motor nerve terminals. The release of low molecular weight amino-compounds may reflect degradation of protein substrates of the synaptic cleft by exopeptidases which liberates amino acids and possibly peptides.

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1199 FUNCTIONAL NEUROMUSCULAR CONTACTS FORMED IN VITRO: AN ULTRASTRUCTURAL AND ELECTROPHYSIOLOGICAL STUDY. Yasuko Nakajima, Yoshiaki Kidokoro* and F. G. Klier*. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907 and Dept. Neurobiol., The Salk Inst., San Diego, CA 92112.

Functional neuromuscular contacts were formed in vitro between rat spinal cord explants and myotubes. At various intervals after the spinal cord explants were added to the myotube-culture (5 hours to 15 days of coculture), the presence of functional neuromuscular contacts was determined by recording miniature endplate potentials (mepps) from myotubes contacted by a few neurites. These myotubes were photographed for identification, and the ultrastructural investigation was conducted only on myotubes having functional neuromuscular transmission.

The presence of mepps was detected as early as 5 hours of co-culture. Although the frequency and amplitude of mepps varied from one myotube to another, a significant increase in the mepp frequency was noticed between junctions formed within 24 hours of co-culture (0.43/sec) and those of 5 to 6 days of co-culture (2.4/sec).

The fine structure of these functional neuromuscular contacts was relatively simple compared to that of adult junctions and showed considerable changes during development. In the earliest stage of functional neuromuscular contacts (7 ~ 8 hour cocultures), a small number of round, clear vesicles and a few dense cored vesicles were already found in the axon endings, but synaptic vesicle aggregation on the prejunctional membrane was found only in a few places. The junctional cleft was narrow, and the junctional membranes did not show specializations except for the occasional presence of coated caveolae in the post-junctional membrane. These results indicate that the presence of synaptic vesicles in the axonal ending is the first noticeable morphological feature for the newly-formed functional contact. A similar structure was also observed in functional contacts formed in 21 ~ 24 hours of coculture, but at this stage some endings accumulated a larger number of vesicles. In the materials of 4 ~ 6 day cocultures, neuromuscular contacts with distinctly more advanced structures were more frequently encountered; number of synaptic vesicles were increased and they were clustered on the prejunctional membrane. These morphological changes are likely to be related to the initial increase in the mepp frequency. However, most of the junctional clefts were still narrow. Only a few contacts had widened clefts with a basal lamina. The neuromuscular contacts formed in 14 day cocultures showed further advanced structures resembling those of adult junctions; the majority had widened clefts with a basal lamina and well-developed postjunctional foldings. (Supported by USPHS grants NS-10457, NS-11918, NS-00102).

1200 THE DIVALENT CATION DEPENDENCE OF SPONTANEOUS QUANTAL SECRETION. R. L. Ornberg* (SPON: H. Gainer). LNNS, NINCDS, NIH, Bethesda, MD 20014.

The effects of black widow spider venom (BWSV), ouabain, 2,4-dinitrophenol, ethanol, and hypertonic saline on the resting miniature endplate potential (mepp) frequency were studied at frog cutaneous pectoris neuromuscular junctions in salines containing varying amounts of Mg^{++} and essentially no Ca^{++}. As previously reported, each of these agents produce a large increase in spontaneous mepps in salines containing little Ca^{++} (10^{-10} M) and millimolar amounts of Mg^{++}. The present study extends these observations to very low Mg^{++} concentrations (10^{-9} M) because it was discovered that tetrodotoxin (2×10^{-6} gm/ml) prevents the adverse postsynaptic effects (spontaneous twitching and membrane depolarization) which ordinarily accompany divalent cation removal.

A number of calcium and magnesium chelators (EGTA, EDTA, CDTA, citrate, and 8-hydroxyquinoline-5 sulfonic acid) were used to buffer Mg^{++} levels in test salines from 3 mM to 10^{-9} M. Ca^{++} was not added to these salines and the calculated free Ca^{++} was 10^{-9} M, assuming a total Ca^{++} concentration of 10^{-6} M due to contamination. In low Mg^{++} saline (10^{-5} M), regardless of the chelator used, the typical rise in mepp frequency produced by BWSV (1 gland/ml), ouabain (0.1 mM) or dinitrophenol (1 mM) is prevented completely. If Mg^{++} or Ca^{++} is added back to the muscle bath at a time corresponding to peak release in normal Mg^{++} (3 mM), an enormous increase in mepps ensues immediately. Preliminary morphological evidence reveals little vesicle depletion before but massive depletion following Mg^{++} addition. In contrast, ethanol and hypertonic saline-induced release are unaffected by low Mg^{++} salines. The rapid onset of high mepp rates following Mg^{++} addition to BWSV, ouabain, and DNP-treated muscles suggests that specific toxic actions involving raised internal Ca^{++} occur even though few quanta are released. Thus, an additional step, involving divalent cations interacting with the surface of the nerve terminal may be required for quantal secretion. Ethanol or hypertonic saline could act on the surface membrane or synaptic vesicles more directly and bypass this step.

1201 ETHANOL AFFECTS THE VOLTAGE-SENSITIVITY AND CALCIUM-DEPENDENCE OF THE RATE OF DESENSITIZATION IN VOLTAGE-CLAMPED EEL ELECTROPLAQUES. Barry S. Pallotta and George D. Webb. Dept. Physiol. & Biophys., Univ. Vermont Col. Med., Burlington, VT 05401.

The effects of calcium, ethanol, and membrane voltage upon activation and desensitization in Electrophorus electricus electroplaques have been studied under voltage-clamp conditions. Cells were equilibrated with 1M ethanol and/or one of several different calcium concentrations for ½h prior to voltage-clamping and bath-application of 0.27 mM carbamylcholine (CCh). The response to CCh is a rapid inward current which peaks and then decays (desensitizes) with a pseudo-first order rate constant k_o.

At -60 mV peak current (I_o) remains constant between 0.5-5 mM Ca^{++} and k_o increases with increasing $[Ca^{++}]$. At 0.1 mM Ca^{++}, however, k_o is fastest, and at 10 mM both I_o and k_o are significantly depressed. Cells treated with ethanol show a reduction in I_o at all $[Ca^{++}]$. Ethanol-treated cells desensitize faster than controls at all $[Ca^{++}]$ except at 0.1 mM, where the effect is a decrease of k_o relative to the control. The ethanol effect is thus $[Ca^{++}]$-dependent.

In physiological saline (2 mM Ca^{++}), k_o is increased 4-fold by increasing membrane polarization over the range of -40 to -90 mV. Ethanol pre-treatment (+2 mM Ca^{++}) causes a doubling of k_o at -40 mV, whereas ethanol increases k_o only slightly at -90 mV. At -40 mV, cells treated with 5 mM Ca^{++} desensitize at the same rate as 2 mM Ca^{++} controls, but at -90 mV I_o is only increased by about 2-fold from the -40 mV rate. In other words, 5 mM Ca^{++} decreases the voltage-sensitivity of k_o so that at -90 mV high calcium inhibits the rate of desensitization.

We have analyzed the interdependence of I_o and k_o in terms of the kinetic model: $A + R \xrightarrow{k_1} AR^* \xleftarrow{k_3}, \xrightarrow{k_4} AD$ where A is agonist, R, receptor, AR* the conducting conformation and AD the desensitized state. This model predicts a decrease in I_o secondary to increases in k_3+k_4 in agreement with our observations. About 15 sec. elapse between the application of CCh and I_o. This time delay is probably due to diffusion of agonist through the 40-80 µm thick layer of gelatinous material covering the electroplaque. Assuming that the apparent k_1 is determined solely by diffusion of agonist, and using equations for diffusion through a plane sheet, we can predict the effects of this unstirred layer on I_o and k_o. As the gel thickness increases, I_o and k_o decrease and time-to-peak current increases. The observed average time-to-peak of 15 sec. predicts a 70 µm thick gel layer, and variations in gel thickness of only ±10 µm may result in variations in I_o and k_o of ±20%. At low agonist concentrations k_1 becomes the rate-determining step and the system becomes insensitive to alterations in the desensitization process (k_3 and k_4). Supported by USPHS ES00885.

1202 MEMBRANE SPECIALIZATIONS IN CULTURED MUSCLE CELLS. H. Benjamin Peng* and Yasuko Nakajima (SPON: Shigehiro Nakajima). Dept. of Biol. Sci., Purdue Univ., W. Lafayette, Ind. 47907.

We have been studying the development of the myotome cells from the Xenopus laevis embryos in tissue culture with the freeze-fracture technique, with special emphasis on the formation of neuromuscular junctions. The muscle cells were isolated from the dorsal mesoderm of stage 21 embryos. After one to two days in culture, cells from the neural tube were added to the culture. Functional neuromuscular junctions were formed one day after the neurons were added as shown by the spontaneous contraction of the muscle cells and MEPPs recorded intracellularly. The preparations were then freeze-fractured with the method demonstrated by Yee et al. (J. Cell Biol. 70: 155a, 1976). Large fracture faces of the plasma membrane, sometimes exposing the whole muscle cell, can be easily obtained.

We have observed the following membrane specializations on the muscle cells. (1) Gap junctions were often observed when two muscle cells make contact with each other. (2) Ruffled membrane patches were frequently observed on the muscle. They appear as a shallow depressions in the plasma membrane with irregular contours and ruffled bottom about 1 µm or longer. Very often a piece of membrane is left on top of such a patch. These results indicate that these patches may be sites of cell-to-cell contact. As many as 30 patches were seen in one cell and they generally lie in a rather localized area. (3) Particle aggregates 0.1 to 0.5 µm wide, consisting of large particles, were often seen on the muscle cell. Their appearance is similar to the peripheral coupling between the plasma membrane and the sarcoplasmic reticulum in some adult muscle. (4) Particle aggregates 1 to 3 µm wide, consisting of tightly packed large particles, were seen on the muscle cell. These large particle patches are often clustered together. The appearance of the particle aggregates resembles post-synaptic particle aggregates observed in adult neuromuscular junctions of various animals. However, these patches seem to exist in a region free of nerve innervation. Therefore, they might represent aggregates of extra-junctional acetylcholine receptors, "hot spots".
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1203 INACTIVATION OF ACETYLCHOLINE RECEPTORS BY LOCAL ANESTHETIC AGENTS. P. Pennefather* and D. M. J. Quastel. Dept. of Pharmacology, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

Tertiary amine local anesthetics split the decay phase of endplate currents at the neuromuscular junction into 2 exponential components. One is faster than the normal decay rate while the other is slower. The model most consistent with our data is that these agents cause a rapid, transient and reversible inactivation of the activated acetylcholine (ACh) receptor-ionophore complex. We have investigated the action of a number of other types of local anesthetics on miniature endplate currents (m.e.p.c.s) recorded at the mouse diaphragm neuromuscular junction. So far all local anesthetics tested have had actions consistent with the above model. Procaine, lidocaine, morphine, naloxone, atropine, scopolamine, quinidine, pentobarbital, diphenylhydantoin, 2-octanol, and menthol all give rise to m.e.p.c.s with a normal height, but with a biphasic decay phase. Streptomycin, an aminoglycoside antibiotic, also had this action. In many cases ethanol was added to the solutions to improve the definition of the m.e.p.c. time course. Ethanol slows the rate of decay of m.e.p.c.s probably by slowing the dissociation of ACh from its receptor. As predicted by the above model, it increased the height of the slow component. Many of the agents decreased the area of the m.e.p.c. without affecting height. Kinetic analysis of the time course of the m.e.p.c. suggests that this represents a slow inactivation process which is dependent on the amount of inactivated receptor produced by fast inactivation. The above model explains how morphine can reduce the size of endplate potentials without affecting the response to iontophonetically applied ACh (Sokoll et al., Prog. Anesthesiol. 1, 169, 1975) and how atropine could appear to act as a non-competitive inhibitor under experimental condition where there was a large receptor reserve (Kirschner and Stone, J. Gen. Physiol. 34, 821, 1951). In the presence of morphine the onset of slow inactivation is about 10 times slower than in the presence of atropine. The rate of onset and offset of fast inactivation also depended on the drug. The action of positively charged agents was voltage-sensitive, while that of negatively charged or neutral agents was not. Studies with varied pH suggest that the positively charged form of morphine is more potent than the uncharged form, while in the case of pentobarbital it is the uncharged form that is more potent.

1204 TENOTOMY DELAYS THE DEVELOPMENT OF SKELETAL MUSCLE MOTOR INNERVATION. Dan A. Riley*. Dept. Anat., Sch. Med., UCSF, San Francisco, CA 94143.

Physiological studies have revealed that the muscle fibers of the rat soleus lose their functional polyneuronal innervation during the second postnatal week and become unineuronally innervated. Furthermore, tenotomy of the soleus prior to the second week slows the normal transition from polyneuronal to unineuronal innervation. The anatomical basis of polyneuronal innervation is multiple axon terminals innervating single muscle fibers; all but one of the multiple terminals per endplate are removed during the second week. The present anatomical study determines whether tenotomy affects the spontaneous elimination of nerve terminals in the soleus muscles of rats and kittens. Soleus muscles of 4-day-old rats were tenotomized and their innervation patterns examined either 9 or 11 days later in silver stained sections. The contralateral soleus muscles and solei from normal rats of the same age served as controls. The tendons had regrown by 9 days, but the muscles were markedly shortened and clearly atrophic. On the average, 36% of the fibers in tenotomized muscles were innervated by multiple terminals, whereas the contralateral and normal muscles both exhibited approximately 30% multiple innervation. By 11 days, the degree of multiple innervation was similar in tenotomized and control muscles. Thus, tenotomy retards the spontaneous elimination of axon terminals from multiply innervated endplates. Other workers have reported that tenotomy of the rat soleus reduces motor neuron activity. In contrast, tenotomy of the cat soleus does not decrease neural activity, although the muscles become shortened and atrophic. If decreased neural activity per se retards neural development, then tenotomy should not delay the process in the kitten. Kitten soleus muscles were tenotomized in 2-wk-old animals prior to the loss of polyneuronal innervation. After 10 days, the tenotomized muscles exhibited 23% multiple innervation while the contralateral control muscles had 16%. Since tenotomy retards neural development in both the kitten and rat, decreased neural activity is unlikely responsible for the delay. In both cases, the tenotomized muscles were smaller than normal and the intramuscular nerve fibers failed to grow in length, as normally occurs in muscles expanding in diameter. Thus, the inhibition of or lack of a stimulus for nerve fiber growth may slow the elimination of excess axon terminals in tenotomized muscles which fail to increase in diameter.

1205 APPEARANCE OF ACETYLCHOLINESTERASE AT CULTURED NEUROMUSCULAR JUNCTIONS. Lee L. Rubin*, Stephen M. Schuetze*, and Gerald D. Fischbach (SPON: U. J. McMahan). Dept. of Pharmacol., Harvard Med. Sch., Boston, MA 02115

Acetylcholine (ACh) receptor clusters and acetylcholinesterase (AChE) are characteristic features of the adult neuromuscular junction. Previous work has demonstrated the appearance of new ACh receptor clusters at sites of transmitter release at embryonic chick nerve-muscle junctions formed in culture. We have now studied the appearance and function of AChE at these newly formed junctions. Synapses between myotubes and motor axons extending from spinal cord explants were located by focal extracellular recording; the spatial resolution of this technique is approximately 5 μm. At each site of transmitter release, the decay phases of extracellularly recorded synaptic potentials were fit by simple exponentials and their mean time constant of decay (τ) was determined. The synapses were grouped into 3 categories based on estimates of τ at 30°C: fast (τ = 1.5 - 2.0 msec), intermediate (τ = 2-3 msec), and slow (τ = 4-6 msec). Cultures were then fixed and stained for AChE by the method of Karnovsky and Roots. Greater than 70% of those synapses at which extracellular potentials showed a fast decay were associated with prominent AChE stain. On the other hand, no reaction product was detected at junctions where τ was greater than 4 msec. Only small faintly stained patches were observed at most synapses exhibiting intermediate rates of decay. These results complement previous studies using AChE inhibitors on adult neuromuscular junctions (Katz and Miledi, J. Physiol. 231: 549, 1973) and directly support the conclusion that hydrolysis of ACh limits the duration of ACh action.

A single myotube may have both AChE-positive and AChE-negative release sites. Thus, the appearance of AChE depends not only on myotube characteristics, but on the properties of particular nerve-muscle contacts. Additionally, at some sites of transmitter release both fast and slowly decaying potentials were recorded. This finding suggests that even within the small field monitored by the extracellular pipette, AChE may have a restricted localization and action.

In contrast to previous studies of nerve-muscle synapses formed by dissociated spinal cord neurons, our data demonstrate the early appearance of AChE at synapses formed by neurites extending from spinal cord explants. AChE was detectable within 24 to 48 hrs after neurite-muscle contact; in older cultures, staining was heavier and more frequent. In these cases, junctions with intermediate decays and light AChE stain may constitute a stage during which AChE is accumulating at particular synaptic contacts. Supported by NS 11160 and the Helen Hay Whitney Foundation.

1206 MODE OF ACTION OF BARIUM IN COUPLING THE NERVE ACTION POTENTIAL TO THE ASYNCHRONOUS DISCHARGE OF ACETYLCHOLINE QUANTA. E. M. Silinsky* (SPONS: R. A. North). Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

It has been observed recently that Ba^{2+} ions are capable of efficiently supporting the quantal release of acetylcholine (ACh) by motor nerve impulses (Silinsky, Brit. J. Pharmac. 59:215 (1977)). In contrast to ACh release in Ca^{2+} solutions, evoked release mediated by Ba^{2+} (i) requires repetitive nerve stimulation at frequencies $\geq$ 1 Hz and (ii) appears as an asynchronous, residual discharge of transmitter quanta that well outlasts the initial stimulation period. This present study was conducted to determine if Ba^{2+} in itself directly couples nerve terminal depolarization to the release of ACh quanta or works indirectly by displacement of Ca^{2+} from internal stores such as mitochondria. The isolated nerve-cutaneous pectoralis preparation of the frog was used in conjunction with conventional intracellular recording techniques. In solutions containing 1.8 mM Ba^{2+} and no added Ca^{2+}, 1 Hz stimulation for < 1 min elevated miniature end-plate potential (min.e.p.p.) frequencies to 5-20 times the control level (seven experiments). As similar results were obtained when a Ca^{2+}-chelating agent was added to the Ba^{2+} solution, it appears that residual extracellular Ca^{2+} is not responsible for mediating evoked release in Ba^{2+} solutions. Ba^{2+} is 2 orders of magnitude more effective than Ca^{2+} in supporting the evoked discharge of min.e.p.p.s - Ca^{2+} in fact is a potent antagonist of the evoked discharge of min.e.p.p.s in Ba^{2+} solutions. This low efficacy of Ca^{2+} appears to render an indirect mechanism whereby Ba^{2+} displaces Ca^{2+} less likely than a direct effect of Ba^{2+} on the release process. The number of ACh quanta released asynchronously by nerve impulses is directly related to the external concentration of Ba^{2+} in a non-linear, possibly exponential, fashion. Mg^{2+} and Co^{2+} both competitively antagonize evoked release in Ba^{2+} solutions. The equilibrium dissociation constant for each ion as an antagonist of asynchronous, Ba^{2+}-dependent release is identical to its respective value as an antagonist of synchronous, Ca^{2+}-mediated release. It thus suggested that Ba^{2+} supports dispersed, quantal ACh release directly by the same release mechanism normally activated by Ca^{2+}. (Supported by NIH grant NS 12782).

1207 SPONTANEOUS AND EVOKED DEPOLARIZATIONS IN PROTRACTOR MUSCLES OF THE OPISTHOBRANCH MOLLUSC, NAVANAX. D. C. Spray, M. Cappell* and M. V. L. Bennett, Dept. of Neuroscience, Albert Einstein Col. Med., Bronx, N. Y. 10461.

Intracellular recordings from muscle fibers that protract the pharynx reveal three depolarizing responses of short duration: (1) spontaneously occurring depolarizations (mPSPs) of one mV or less; (2) PSPs of a few mV evoked by stimulation of the nerve, and (3) action potentials seen during penetration and evoked by depolarization of the fiber. The mPSPs can exhibit amplitude, rise time, and interval distributions similar to those of miniature end plate potentials of vertebrate junctions with localized innervation. The PSPs are about six times the mean amplitude of the mPSPs. In many fibers, amplitude and rise time are not affected by increasing the nerve stimulus strength above threshold; in some fibers both amplitude and rise time increase abruptly as strength of the stimulus is increased. The second case, so far only seen in the dorsal protractor, probably reflects multifiber innervation either of single or of electrotonically coupled fibers. PSP amplitude is depressed by curare and high Mg++ and enhanced by prostigmine and high Ca++. Curare also abolishes the mPSPs. Action potentials are generated in muscle fibers by intracellular depolarizations of 20-30 mV. Protractor muscles of a large specimen may reach a length of 5 cm or so, and action potentials may serve to spread contraction along the muscle. Ventral protractor muscles are innervated by a motoneuron in the ipsilateral buccal ganglion; dorsal protractor muscles are innervated by two motoneurons, one in each buccal ganglion. Stimulation of a protractor motoneuron elicits PSPs in the muscle fibers similar to those evoked by nerve stimulation. We conclude that neuromuscular transmission and postsynaptic responsiveness can be very similar in molluscan, arthropod, and vertebrate muscle.

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1208 ETHANOL ON THE CRAYFISH NEUROMUSCULAR JUNCTION. Staiman, A. L. Acosta-Urquidi, J.*, Carlen, P. L. and Corrigall, W. A. Neurobiology Lab, Addiction Research Foundation, Toronto, Ontario, Canada. M5S 2S1

The effects of ethanol on the vertebrate cholinergic neuromuscular junction are well documented. To examine whether ethanol acts in a similar fashion at other neuromuscular synapses we studied the effect on the leg-opener neuromuscular preparation of the crayfish. Selective stimulation of the excitor axon produces an excitatory junctional potential (EJP) mediated by glutamate. Stimulation of the inhibitory axon produces an inhibitory junctional potential (IJP) mediated by GABA. Stimulus frequency used was 5/sec. Ethanol depressed both the EJP and the IJP. At concentrations of 0.2M, 0.4M and 0.6M the EJP was depressed to approximately 80%, 60% and 40% of the control amplitude respectively. Similar results were found with the IJP and therefore there appeared to be no difference in the sensitivity between the EJP and IJP to ethanol. The nerve terminal spike amplitude and latency was unaffected by the former concentrations. Input resistance was measured using two microelectrodes. Depression of the input resistance paralleled the depression of the EJP and IJP. Therefore, the EJP and IJP depression was due to the effect of ethanol on the input resistance. Concentrations of ethanol from 0.2M to 1M caused a small and reversible depression of the resting membrane potential, between 1 to 6mV. At higher concentrations (from 0.8 to 1M) and at approximately 50% reduction of the EJP or IJP hyperexcitability of the nerve terminal was produced. One stimulus to the axon produced from 2 to 10 synchronous EJP's or IJP's. Using a focal extra-cellular electrode it was observed that this effect was due to multiple spiking at the nerve terminal. The effect of ethanol on the spontaneous release of miniature excitatory junction potentials (mejp) was studied. At lower concentrations (0.2M and 0.4M) there was no detectable increase in mejp frequency. At higher concentrations (0.6M to 0.8M) where the input resistance is significantly depressed there also appeared to be no increase in the frequency of mejp's.

(supported by Addiction Research Foundation/Medical Research Council of Canada)

1209 EVIDENCE THAT BETA-BUNGAROTOXIN BINDS DIRECTLY TO NERVE TERMINALS: SEPARATION OF BINDING AND ENZYMATIC ACTIVITY. Peter N. Strong, Randall J. von Wedel*, Regis B. Kelly. Dept. Biochem. & Biophys., U. Cal., San Francisco, CA 94143.

ß-Bungarotoxin (ßTX), the presynaptic neurotoxin isolated from the venom of the krait, Bungarus multicinctus, modifies release of acetylcholine at mammalian and amphibian neuromuscular junctions. There is accumulating evidence to suggest that ßTX's physiological effects at the synapse are mediated by the toxin's endogenous phospholipase A_2 activity. The precise cause of the toxin's highly selective action is unknown; ßTX could 1) bind to a specific receptor on nerve terminal plasma membranes and hydrolyze phospholipids in the vicinity, or 2) selectively hydrolyze a unique phospholipid substrate on nerve terminals.

We have sought to distinguish between these two possibilities by looking for evidence of toxin binding under conditions of enzyme inactivation. ßTX phospholipase activity was blocked in two distinct ways. In the first approach we examined the ability of phospholipase inactivated ßTX (chemically modified with *p*-bromophenacyl bromide, BPB-ßTX) to protect a frog neuromuscular preparation against native toxin. 1 µM BPB-ßTX had no effect on indirectly stimulated muscle contraction in Ca^{++} Ringer. After extensive washing, an application of 50 nM native toxin gave no reduction in tetanic tension for 45 min, a time which gave >95% inhibition in control preparations.

In the second approach, we have examined the effects of toxin in Ringer solutions in which Ca^{++} was replaced by Sr^{++}, since Sr^{++} inhibits the phospholipase activity of ßTX. After 30 min in toxin, the preparation was washed extensively for 120 min with Sr^{++} Ringer. Subsequent addition of Ca^{++} Ringer (no toxin) resulted in contraction failure analogous to that seen when native toxin was added to a muscle in normal Ca^{++} Ringer.

These experiments suggest that when the phospholipase activity of ßTX is inactivated either by chemical modification or by Sr^{++} the toxin will still bind to nerve terminals. If inactive derivatives bind, then it is reasonable to assume that a presynaptic ßTX receptor exists. Inactive derivatives, suitably labelled, offer potential as biochemical markers of presynaptic nerve terminals. Supported by NIH grant NS09878. PNS is a Muscular Dystrophy Assn. Postdoctoral Fellow.

1210 EFFECT OF MORPHINE AND NALOXONE IN NEUROMUSCULAR TRANSMISSION. Philip C. Su and Arthur D. Rosen. Neuro. Svs., VA Hosp. Northport, NY and Div. of Neuro., State Univ. of NY at Stony Brook, NY

Morphine has been shown to have both facilitative and depressive effect on neuromuscular transmission. Naloxone auguments the depressive effect of Morphine. We have studied the quantal release produced by Morphine and Naloxone in neuromuscular transmission. Our results indicate that Morphine increases the acetylcholine quantal release in low concentration and reduces the quantal release in high concentration. In addition, we were able to demonstrate that Naloxone specifically antagonizes the facilitative effect of quantal release of Morphine.

Mice phrenic nerve-diaphragm and frog cutaneous pectoris nerve-muscle preparations were used in vitro. Endplate potential (EPP) were recorded in curarized preparations or high magnesium solution. Quantal content was determined by either the ratio of mean amplitude of EPP's and miniature endplate potentials (MEPP) or by the variance method of EPP's. Corrections for nonlinear response of EPP were made by assuming equilibrium potential of 15mV inside negative. At concentration of $1x10^{-6}$ g/ml to $1x10^{-5}$ g/ml Morphine increased the quantal content. This facilitation effect is most noticeable in the first 60 min. after perfusion with Morphine. At concentration of $1x10^{-4}$ g/ml the quantal content was significantly reduced. Morphine has no effect on immediate available store, mobilization rate or probability of release of acetylcholine in the concentration of $1x10^{-6}$ g/ml to $1x10^{-4}$ g/ml. Naloxone at the concentration of $1x10^{-5}$ g/ml completely abolished the facilitative effect of Morphine on quantal content. Naloxone alone ($1x10^{-5}$ g/ml to $1x10^{-6}$ g/ml) had no effect on the quantal content. Morphine at $1x10^{-5}$ g/ml to $1x10^{-4}$ g/ml reduces the amplitude of MEPP in the range of 20% to 80%. Naloxone ($1x10^{-4}$ g/ml) also reduced the amplitude of MEPP about 30%, and did not antagonize the effect of Morphine on the amplitude of MEPP. Both Morphine and Naloxone produced no significant effect on the frequency of MEPP.

We conclude that Morphine and Naloxone in high concentration have significant postsynaptic effect on the neuromuscular junction which is probably nonspecific since Naloxone does not antagonize the effect of Morphine. However, the presynaptic facilitative effect on quantal release produced by Morphine is abolished completely by Naloxone. We believe the neuromuscular junction is a useful simple model for the study of mechanism of action of Morphine at a molecular level.

Supported by Veterans Administration General Research Fund.

1211 MECHANISMS REGULATING ACETYLCHOLINE SYNTHESIS AT THE NEUROMUSCULAR JUNCTION. Ken Vaca* and Guillermo Pilar (SPON: M. Wilson). Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268.

Electrical stimulation of the chick ciliary nerve causes a frequency-dependent increase in the high affinity uptake of ^{3}H-choline (Ch) and its conversion to acetylcholine (ACh) in the terminals innervating the iris muscle. Conditioning trains of stimuli have little or no effect on uptake or synthesis during subsequent periods of rest, suggesting that after ACh is hydrolyzed in the synaptic cleft, Ch is rapidly and efficiently reaccumulated to replenish ACh stores. If the tissue is depolarized with high K^+ or veratridine during the incubation with ^{3}H-Ch, accumulation is substantially reduced, even in high Mg^{++}, 0 Ca^{++} medium. Thus, energy derived from the membrane potential contributes to the driving force for Na^+-coupled Ch transport. The role of the Na^+ gradient was evaluated in experiments where Na^+, K^+-ATPase was inhibited with either ouabain or K^+-free medium for various lengths of time prior to the uptake of Ch. In both cases, there was a rapid phase of inhibition, which may be due to a direct effect on membrane potential and a prolonged slower phase which could be fit by an exponential curve; presumably reflecting dissipation of the Na^+ gradient. If subsequent to depolarization with high K^+, the tissue is returned to normal medium containing ^{3}H-Ch, the V_{max} for transport is doubled (from 4.9 to 9.8 pmoles/8 min/iris) while the V_{max} for ACh synthesis increases about five-fold (from 1.2 to 6.3 pmoles/8 min/iris) without any significant change in K_m values. The influxes of both Na^+ and Ca^{++} during depolarization are involved in the subsequent activation of uptake. Replacement of Na^+ with either Li^+ or sucrose or addition of tetrodotoxin reduces the extent of activation. Omission of Ca^{++} or antagonism of its influx with D600 or high Mg^{++} has a similar effect. If ouabain is present during Ch uptake following preincubation in high K^+, uptake is not significantly increased; however, ACh synthesis is substantially elevated. It is proposed that activation of Ch uptake is due in part to an electrogenic Na^+ pump. Equations derived using the principles of nonequilibrium thermodynamics allow us to calculate that a 10 mV hyperpolarization will more than double the ability of the terminals to accumulate Ch. In order to account for the differential effects on uptake and synthesis, it is hypothesized that the Ca^{++}-dependent release of ACh from a compartment, presumably vesicular, results in an accelerated translocation of ACh into that compartment from the cytoplasm thus driving ACh synthesis by mass action and allowing further accumulation of Ch.
Supported by NIH-NS 10338 and the Univ. of Connecticut Research Foundation.

1212 PERMEABILITY OF FROG END-PLATE MEMBRANES TO ORGANIC AND INORGANIC CATIONS. Shigenori Watanabe* and Toshio Narahashi (SPONS: S.-C. Cheng). Dept. Physiol. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710 (Present address: Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611).

Permeability of the end-plate membrane of the frog sartorius muscle to a variety of organic and inorganic cations has been studied by iontophoretic applications of acetylcholine (ACh). Recording of the membrane potential was made from the end-plate region by means of an intracellular microelectrode filled with 3 M KCl solution, while ACh or carbachol was iontophoretically applied to the external surface of the end-plate membrane via a microelectrode filled with 1 M ACh or 1 M carbachol. For study of a test cation, sodium in Ringer's solution was replaced by an equimolar concentration of the test cation. Four organic cations caused a sizable increase in ACh potential, with the peak amplitude reaching 150-250% of the control in ammonium-substituted Ringer's solution, 120-160% in formamidine, 120-180% in methylamine, and 150-220% in hydrazine. The resting membrane potential was only negligibly decreased by these cations except for ammonium which caused a 5-20 mV depolarization. Guanidine and lithium decreased the amplitude of ACh potential to 80-90% of the control. Methylguanidine was unique in that it suppressed the ACh potential at low concentrations with an apparent dissociation constant of 0.2 mM. This effect was reversible upon washing with methylguanidine-free medium. Choline has a similar but weaker blocking action on the ACh potential, with 5 mM choline decreasing the potential to 20-30% of the control. It also caused a slight depolarization. The time course of the ACh potential was affected by some of the cations. The time to peak amplitude and the half-decay time were prolonged by methylamine, hydrazine, guanidine and lithium. Since carbachol iontophoretic application yielded the same results as ACh application, and also since physostigmine prolonged the falling phase of ACh potential but did not potentiate the peak amplitude, the observed increase in the peak ACh potential by certain cations is not due to the inhibition of acetylcholinesterase. Thus, ammonium, formamidine, methylamine and hydrazine are more permeant than sodium to the end-plate membrane during the transmitter action. Guanidine and lithium are only slightly less permeant than sodium. The relatively non-selective permeability of the end-plate membrane to these cations is reminiscent of ionic channels of the resting squid axon membrane, but the potent blocking action of methylguanidine and choline is not observed in the squid axon membrane.
(Supported by NIH grant 14145).

1213 POSSIBLES ROLES OF TWO SUBPOPULATIONS OF ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES IN THE PATHOGENY OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA GRAVIS. Anne D. Zurn* and Bernard W. Fulpius. Dept. Biochem., University of Geneva, Sciences II, Geneva, Switzerland.

Two different subpopulations of anti-acetylcholine receptor (AcChR) antibodies were studied during the evolution of experimental autoimmune myasthenia gravis (EAMG) in a rabbit immunized with Torpedo AcChR.

The antibodies directed against sites other than the bungarotoxin (Bgt)-binding site of the receptor were quantified with a radioimmunoassay. At the onset of paralysis, the titer of these antibodies was 7 nmol ^{125}I-α-Bgt-AcChR complex precipitated per ml serum, a value only 1.4 times higher than the highest level measured before.

The anti-AcChR antibodies directed against the toxin binding site of the AcChR (toxin from Naja naja siamensis) were measured with an assay based on the inhibition of α-toxin binding to the soluble AcChR. At the onset of paralysis, the amount of inhibition was 37% per 0.1 µl serum, a value seven times higher than the highest level measured before paralysis.

When portions of mouse diaphragms were incubated successively with control serum and ^{125}I-α-toxin, the receptors within the region of the nerve terminals were radioactively labelled. With anti-AcChR serum taken at the onset of paralysis, only background radioactivity was detected in this region.

We propose that only one of the two populations of anti-AcChR antibodies, the one which inhibits toxin binding, might be involved in the pathogeny of the neuromuscular blockade observed in EAMG.

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NEURONAL CIRCUITS AND PATTERN GENERATION

1214 TRIGGERING OF EXPIRATORY NEURONS IN COUGH. H.L. Batsel and A.J. Lines, Jr. Medical Research Programs VAH Long Beach, Ca 90822.

In lightly pentobarbitalized cats, weak electrical stimulation of the cervical vagus at high frequency (300 Hz) activates about half of the inspiratory neurons located ipsilaterally in ventrolateral nucleus of tractus solitarius (NTS). Under the same conditions about half of the expiratory neurons located ipsilaterally in nucleus retroambigualis (NRA) is inhibited. Slightly stronger stimulation of the vagus is able to increase the number of inhibited expiratory neurons, including a few in contralateral NRA. The effect of such weak stimulation of the vagus on respiratory movements resembles the inflation reflex. Stimulation of the vagus at parameters which cause cough (17 Hz and 5 times the weak stimulus) beginning during expiration results in an initial inhibition of expiratory neurons followed by a long preparatory inspiration, followed in turn by high-frequency bursting discharge of the expiratory neurons which earlier had been inhibited. Stimulation of the vagus at cough parameters causes fixed latency driving of about 80% of the inspiratory in ipsilateral NTS. We conclude that about half of the NTS inspiratory neurons activated by weak high-frequency stimulation of the vagus are interneurons whose function is to promote a shift to inspiration as a preliminary to cough. We assume that the transient parallel inhibition of expiratory neurons must somehow favor the development of the preparatory inspiration.

1215 AUTORADIOGRAPHIC TRACING OF DORSAL CENTRAL GRAY PROJECTIONS IN THE RAT. S. Browder*, D. C. German, M. Mendershausen*, R. T. Brown*, R. S. Kiser, and G. A. Mihailoff (SPON: R. Seaman). Depts. of Physiol., Psychiat., and Cell Biol., U. of Texas Health Sci. Ctr., Dallas, TX, 75235.

The dorsal central gray (DCG) area has been linked with the paleospinothalamic pain system, and electrical stimulation here in animals produces behavioral agitation with frantic running, urination and defecation (Kiser & Lebovitz, Physiol. & Behav., 15 (1975) 47-53). Stimulation of this region in man produces a complex mixture of affective, autonomic, and painlike sensations with emotional responses of fright and feelings of impending death (Nashold et al., J. Neurosurg., 3 (1969) 14-24). In order to study the anatomical projections of this area in the rat, tritiated amino acids (leucine and/or proline, 27-43 uCi/ul) were pressure injected (0.2 µl over 30 min.) or microiontophoresed into the DCG of 10 animals. With the microiontophoretic method, very small injection sites were made within DCG loci where electrical stimulation produces aversive behavioral effects. All projections were primarily ipsilateral to the injection site. Ascending and descending projections were observed running within the central gray. The descending projections travelled to the caudal pons within the lateral reticular formation with label found throughout the brainstem reticular formation. From the injection site, labelled fibers radiated dorsal, lateral and ventral into the tectum in Wisschedels' radiato grisea tegmenti. Ascending axons were followed into the lateral habenular nucleus, parafascicular nucleus of the thalamus, and ventrobasal thalamus. Labelling was also found in the posterior hypothalamic area. These results are consistent with previous autoradiographic studies in the cat, and they will be discussed with respect to silver degeneration studies of DCG projections in the rat.

(Research supported by NIMH Grants MH-27574 and MH-26032).

1216 NEURAL MECHANISMS THAT ENCODE THE INTENSITY AND THE MODALITY OF CUTANEOUS PRESSURE AT THE NEURONS OF THE CAT MEDULLA AND THALAMUS. R. Emmers. Department of Physiology, College of Physicians & Surgeons, Columbia University, New York, N. Y.

When single electrical pulses are substituted for pressure applied to a small peripheral field on the cat's tongue to drive a neuron in the nucleus VPM of the thalamus, the firing pattern of the neuron has two activity peaks. These can be seen best on a post-stimulus histogram. The first has a latency of 6-12 msecs and its height varies with the intensity of stimulation (I peak); the second, with an avg latency of 40 msecs, is characteristic of those neurons that respond to pressure by tonic activity throughout the period of stimulation. This peak, therefore, is the code for a certain sensory modality (M peak). (Brain Res. 21:91, 1970). When dual electrical pulses are applied to the same field, the I-M pattern does not become disorganized but it undergoes certain predictable changes. 1) The 2nd stimulus of the pair is unable to fire the thalamic neuron at interstimulus intervals of 25-30 msecs. Consequently, the pattern originated by the 1st stimulus is disturbed only for as much as the 2nd stimulus interrupts the spontaneous activity of the system by activating inhibitory interneurons at the medullary level. 2) The effectiveness of the 2nd stimulus to fire the thalamic neuron increases progressively as the interstimulus interval is lengthened. With the I peak of the 2nd stimulus (I_2) increasing in height, the M peak of the I_2 also appears (M_2). As a result, the activity peaks interlace at some interstimulus intervals (I_1, I_2, M_1, M_2) but are separate at others (I_1-M_1, I_2-M_2). Interlacing of the activity peaks is controlled by an inhibitory period that occurs at the corresponding pressure relay neurons in the trigeminal nuclear complex soon after the neuron fires for an I peak. The inhibition dissipates slowly within 160 msecs. Consequently, firing of the trigeminal neuron forms only I peaks. In fact, firing of the trigeminal neurons was at its lowest at the time when the thalamic neurons fired for the M peaks. Therefore, it was concluded that the M peaks are formed by re-excitation of the thalamic neurons via circuits that originate on the thalamic level and that the inhibitory period at the trigeminal neurons prevents the modality peaks of multiple stimuli to merge. (Aided by grant NS-03266 from NINCDS)

1217 RHYTHM GENERATION IN NEURONAL NETWORKS WITH RECURRENT CYCLIC INHIBITION. W. Otto Friesen and Gunther S. Stent*, Dept. Mol. Biol., Univ. California, Berkeley 94720.

A network of intra- and intersegmentally connected interneurons gives rise to the swimming rhythm in the medicinal leech (Friesen, W., Poon, M. and Stent, G., PNAS 73, 3734-3738, 1976). The origin of the oscillations in this network can be explained by the mechanism of recurrent cyclic inhibition first proposed by G. Szekely (Acta Physiol. Acad. Sci. Hung. 27, 285-289, 1965). This mechanism applies to any neuron ring consisting of an odd number of cells in which each cell provides inhibitory input to and receives inhibitory input from one other cell, and where all cells are supplied with some tonic source of excitation. Activity progresses around this ring in a sense opposite to the direction of the inhibitory connections. The cycle period of such a network depends on the sum of the times required for each cell to reach impulse threshold following cessation of its inhibition. Further properties of the mechanism of recurrent cyclic inhibition were explored through theoretical considerations and modeling with analog electronic neuronal elements (neuromimes). These neuromimes are "integrate and fire" analog models with provisions for excitatory and inhibitory synaptic inputs, threshold control and impulse outputs. In modeling experiments all neuromimes received externally controlled tonic excitatory inputs from a common source. Experiments showed that in neuromime rings with three or five elements, in which each element inhibits the next with no conduction delay, the cycle period of the oscillations depends almost exclusively on the summed inhibition recovery times of the elements. Since the recovery times vary inversely with the tonic excitation levels, the cycle period of the oscillations also varies inversely with the level of excitation. When a delay was introduced in the impulse conduction between two of the elements in an inhibitory ring, the cycle period was found to display a constant time sector in addition to the variable time sector, in agreement with physiological data from swimming leech preparations. The introduction of the conduction delay, which was designed to mimic the approximately constant time required for an impulse to traverse the distance between segmental ganglia in the leech nerve cord, also provided an adequate phase delay between activity in neuromime elements modeled to be in different ganglia. Finally, the concatenation of two five-membered rings to model the interconnections of three ganglionic oscillators gave rise to an increased cycle period at constant excitation levels and provided for proper phase delays for the modeled activity in a chain of ganglia. (Supported by NIH grants GM 17866 and NS 12818, NSF grant BNS 74-24637 and NIH postdoctoral fellowship NS 05144).

1218 NEURONAL ORGANIZATION OF ESCAPE SWIMMING IN TRITONIA. Peter A. Getting* (SPON: D. H. Perkel). Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305.

Escape swimming behavior in Tritonia diomedea consists of two major components: an initial reflexive withdrawal followed by a series of alternating ventral and dorsal flexions. The basic mechanism of generating motoneuronal activity "switches" from reflexive to centrally programmed. Three classes of cerebral interneurons and some of their synaptic connections have been identified. Reflexive withdrawal interneurons (RWI) receive direct input from sensory afferents and synapse with motoneurons in the ipsilateral pedal ganglion. This class of interneurons is excited during reflexive withdrawals but is inhibited during swimming. A second class of interneurons termed swim interneurons (SI) also receive excitatory input from sensory afferents and synapse with ipsilateral pedal motoneurons. The SI, however, are excited during both reflexive withdrawals and swimming during which the SI burst in phase with the dorsal flexion motoneurons. The third set of interneurons (C-2) are necessary for the normal initiation and maintenance of the cyclical swim phase. Activity in C-2 neurons appears to retrigger a swim oscillator network cycle-by-cycle. Motoneuronal activity during reflexive withdrawals is determined by the summation of inputs from at least three sets of identified interneurons (RWI, SI, C-2). During swimming, C-2 neurons inhibit the RWI such that the motoneurons are freed to respond primarily to inputs from the SI and C-2 neurons. The switch from reflexive to programmed motor output involves a reordering of central circuitry

1219 MOTOR PROGRAM FOR LOCOMOTION IN APLYSIA DOES NOT REQUIRE PERIPHERAL FEEDBACK. W. Hening, T. Carew and E. Kandel. Div. Neurobiol. & Behav., Dept. Physiol., Columbia P&S, New York, N.Y. 10032.

The locomotor pattern of a number of invertebrates has been shown to result from a central program. In view of the advantages of Aplysia for a cellular analysis of behavior, we have examined the nature of the neural mechanisms underlying pedal locomotion. We now report a preliminary analysis of walking in Aplysia and show that the central neuronal generator for walking produces patterned output without the necessity of afferent feedback.

By videotape analysis of intact animals, we confirmed that walking consists of stereotyped cyclical activity of the foot, parapodia and tail. To find motor neurons participating in this behavior, we recorded from cells in the pedal ganglia of semi-intact preparations. We identified several populations of presumptive motor neurons acting upon the foot, parapodia and tail. Since the semi-intact animal shows walking behavior, we were able to correlate the activity of identified neurons with the phases of walking by simultaneous videotape and intracellular recording. We found that motor neurons are active in those phases of walking during which their target organs exhibit characteristic activity. To determine whether the central generator for walking requires afferent feedback, we conducted two types of experiments. First, while maintaining penetrations of identified cells, we made nerve sections to isolate the ring ganglia (pedals, pleurals and cerebrals). After sectioning, typical cyclical activity persisted in the presumptive motor cells. Second, we recorded from identified presumptive motor neurons in the isolated nervous system. We found that these cells show cyclical activity, both spontaneously and following stimulation of a peripheral nerve, that was indistinguishable from that of walking in the semi-intact preparation. We therefore concluded that the neural generator for walking is located in the ring ganglia and that its patterned output does not require afferent feedback.

We have also examined how parapodial motor neurons contribute to different behaviors. These motor neurons are active both in walking and in respiratory pumping, a complex behavior whose command elements are located in the abdominal ganglion. As in other Aplysia motor systems with graded output, these motor neurons are organized in parallel; they receive both common and unique synaptic input, but form no synaptic interconnections. As a result, different subsets of motor neurons can be selectively activated to produce the varying patterns of activity required for different behavioral sequences.

1220 DEVELOPMENT OF SYNAPTIC CONNECTIONS IN THE HIPPOCAMPUS FOLLOWING REMOVAL OF NORMAL RECIPIENT NEURONS BY NEONATAL X-IRRADIATION. Anders Hjorth-Simonsen* and Søren Laurberg* (SPON: Erik Westergaard). Dept. Anat., Univ. Aarhus, DK-8000 Aarhus C, Denmark.

The brains of new-born rats were exposed to graduated X-irradiation, which arrests further proliferation of neurons while it is relatively harmless to differentiating or mature neurons. The majority of the granule cells, which form a discrete layer in the dentate gyrus, develop after birth in a latero-medial sequence, consequently, in the irradiated animals, only the lateral portion of the granule cell layer is formed. The major sources of afferents to the granule cells, viz., the medial and lateral entorhinal cortices and the regio inferior of Ammon's horn, are generated before birth and, therefore, would be expected to be resistant to the irradiation. The axons from the three different sources normally terminate, with little or no overlap, in specific zones across the granule cell dendrites. These zones are reflected in a distinct chemoarchitectonic stratification seen in Timm-sulfide silver preparations.

In the irradiated rats that were allowed to grow up, an equally distinct stratification was found with the Timm method not only above the surviving granule cells, but also medially where these were lacking. It was found with the Fink-Heimer method that each histochemically defined zone corresponded to one of the three major afferent systems to the dentate gyrus in the normal sequence from the pial surface. These findings indicate that the spatial segregation of the converging pathways has occurred relatively independent of the normal target neurons at the site examined.

Golgi preparations of irradiated brains showed that the basal dendrites of pyramidal cells in the Ammon's horn adjacent to the hilus of the dentate had extended into the terminal zones formed in the absence of granule cells. It is possible that the abnormal dendritic growth has been induced by the afferents deprived of normal target cells.

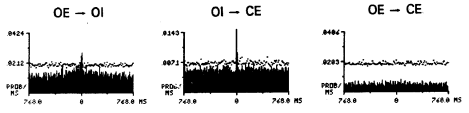
1221 SYNAPTIC CHANGES AND MODIFIED BEHAVIOR IN A CHRONICALLY ISOLATED SEGMENT OF THE MEDICINAL LEECH. William B. Kristan, Jr., Department of Biology, University of California, San Diego, La Jolla, California 92093

Swimming in the medicinal leech is a well-defined behavioral act. After initially flattening, each of the 21 mid-body segments undergoes an alternating dorsal and ventral longitudinal muscle contraction in metachronal synchrony with the other segments. If all the interganglionic connectives to a single segmental ganglion are severed, the neurally isolated segment so produced is at first incapable of swimming movements. However, after 1 to 2 weeks of isolation, the segment acquires the ability to produce swimming movements by itself. In order to find the basis for this behavioral change, properties of neurons known to be involved in swimming were measured in ganglia isolated for 2 to 4 weeks. Three kinds of synaptic connections are found in these ganglia which are never seen in normal segmental ganglia: electrotonic synapses between dorsal and ventral longitudinal muscle excitors; inhibitory connections from excitatory motor neurons onto inhibitory motor neurons; and connections from tactile sensory neurons onto a cell which initiates swimming. The latter synaptic modification is potentially involved in the acquisition of the ability of the isolated segment to swim.

INTERACTIONS AMONG CRAYFISH CLAW MOTOR NEURONS DURING ACTIVE AND PASSIVE DACTYL MOVEMENTS. B. G. Lindsey and G. L. Gerstein. Dept. Physiology, Sch. Med., Univ. Pa., Philadelphia, Pa. 19104.

Spike trains of 3 crayfish claw motor neurons, the opener excitor (OE) and inhibitor (OI) and the slow closer exciter (CE), have been monitored simultaneously 1) while the dactyl is held motionless, 2) during imposed dactyl movements (reflex loop open: motor neuron axons cut distal to peripheral electrodes), and 3) during movement playback experiments. In the latter, neural activity and dactyl position were recorded during active ("spontaneous" or reflexively induced) movements. The reflex loop was then opened and the position signal played back into a galvanometer. This replicated the previous movements produced by the intact crayfish; neural activity was monitored again. Statistical analyses of the spike trains reveal that OI can be an element of more than one functional group of neurons: OI may be positively correlated with both OE and CE while OE and CE may or may not show signs of coordinated firing (Fig.). Results of playback experiments show that OI and OE can be positively correlated during active opening while the probability of CE activity is relatively low; intermittent imposed movements during this phase of the experiment show that CE can respond to proprioceptive inputs. During the playback phase OI and CE spike trains are usually temporally correlated while OE may be virtually silent. Intracellular recordings of OI and CE made while simultaneously monitoring claw joint receptors have revealed that 3 types of receptors (differentiated by responses to opening step movements of dactyl) excite both efferents. The sensitivities of these receptors are not under any known direct efferent control; therefore, movement playback technique helps characterize the contributions of central mechanisms in modulating sensory-motor interactions. In the cases in which active movements are "spontaneous", the sensory information transmitted to the CNS should in principle be the same during both movement and playback phases of the experiments.

Fig.: Cross correlograms (reference event first). Spike trains recorded simultaneously during imposed opening movements of dactyl (2-29°, 3°/sec, 26 repetitions). Number of events: OE, 318; OI, 1595; CE, 1265. Normalized ratio, events in peak/events in "background": 1.0, 1.1, 0. Control calculation (target events shifted by one stimulus cycle) raised by arbitrary amount for clarity. (NIH 1F32 NS05650-01; NS05606)



1223 ANALYSIS OF NEURAL NETWORK COMPONENTS USING SINGLE CELL ISOLATION. J. A. London* and M. B. Merickel (SPON: C. S. Carter). Dept. of Physiol. and Biophysics and Bioengineering Program, Univ. of Illinois, Urbana, IL. 61801.

Analysis of simple neural networks is often complicated by interactions between component neurons as well as by specialized cellular properties. This laboratory is approaching the problem by studying the properties of isolated neurons of the buccal ganglion of the snail, Helisoma trivolvis, using a technique based on Kostenko's method of cell isolation (Kostenko, et al, Comp. Biochem. Physiol., 1974, 49A, pp. 89-100). The neural sheath is softened by soaking the ganglion in a trypsin-hyaluronidase solution (0.25%, for 7 minutes, 25°C). Intracellular recordings taken from the same cell before and after treatment showed no change in characteristic firing patterns, action potential height and waveform. The neural sheath was then teased away using electrolytically sharpened needles, allowing the cells of the ganglion to float free of the ganglionic mass. These cells maintained characteristic synaptic activity, firing patterns and membrane properties (e.g. action potential height and width, time constant). Complete isolation was performed by cutting the axon of the cell from the soma. The larger cells of the ganglion could be followed throughout the isolation procedure visually. Large protractor motoneurons which are characterized by a membrane hyperpolarization usually followed by a burst of spikes and which are electrically coupled when in the intact ganglion showed none of these characteristics when isolated, but would still generate action potentials when stimulated. Increases in membrane time constants were measured which are consistent with the cell being "unloaded" from the rest of the network.

This technique is being used to study the mechanisms by which a network of electrically coupled neurons in the buccal ganglion (i.e. cyberchrons, Kater, S. B., Amer. Zool., 1974, 14(3), pp. 1017-1036) generates rhythmic bursting output. It has been difficult to determine whether burst generation in this network is a property of individual cyberchron neurons or an emergent network property. Dyes injected into these small cells can be used to follow specific cells through the isolation procedure. This will allow a direct comparison of cellular properties before and after isolation and observation of these cells' intrinsic electrical activity.

1224 RATE-SPECIFIC AND STATE-SPECIFIC ASPECTS OF SPONTANEOUS DISCHARGE PATTERNS IN CAT LATERAL GENICULATE UNITS. Robert W. McCarley and Odile Benoit*, Harvard Medical School and University of Paris, France.

The spontaneous discharge pattern of cells in thalamic relay nuclei is usually described in "state-specific" terms: they tend to discharge in bursts in synchronized sleep and with isolated spikes in waking (W) and desynchronized sleep (D). An alternative hypothesis is that discharge rate determines pattern. The cause of the burst pattern is unknown but juxtacellular microelectrode recordings in the lateral geniculate body (LGB) suggest that each burst can be viewed as a single event, a postsynaptic potential whose time of occurrence is marked by the first spike in the burst. The isolated spikes and the first spike in each burst can thus be characterized as the "primary events", and it is a logical first step to begin the analysis of rate-pattern relationship in the LGB with these primary events. The variance, third, and fourth moment are used as the first order pattern measures; they are directly related to aspects of histogram form and are also mathematically justified as the first three terms in a power series approximation of the density (histogram) function. First order measures are those not dependent on the serial order of intervals.

The data presently analyzed is from extracellular recordings of 10 histologically localized LGB units during spontaneously occurring episodes of W, synchronized sleep with spindles, synchronized sleep without spindles, and D. Several recordings of each unit in the same state were included in the analysis since one of the purposes of the study was to examine the rate-pattern relationships in one cell under different conditions of rate and state, as well as between cells.

A least squares linear regression analysis was done on stationary records to determine the relationship between the mean interval duration ($\overline{ISI}$) and the pattern measures of the primary events using the equation: $\text{Log } Y = A_0 + A_1(\text{Log } \overline{ISI})$ where Y was successively taken to be variance, 3rd moment and 4th moment. The percentage of variance explained was 96.4% for the variance and 91.6% and 91.8% for the 3rd and 4th moments. Inspection of the plots of the pattern measures showed no apparent difference in goodness of fit to the above regression line in comparisons of recordings of different cells and from one cell under conditions of different rates and states. These data suggest that for primary events discharge rate determines first order discharge pattern in spontaneous activity of lateral geniculate cells.

1225 MEMBRANE PROPERTIES OF BURSTING NEURONS OF ELECTRICITY COUPLED NETWORK INVESTIGATED WITH SINGLE ELECTRODE VOLTAGE CLAMP. Michael B. Merickel. Bioengineering Program and Dept. Physiol. and Biophysics, U. of Ill., Urbana, IL 61801.

The cyberchron network in the snail Helisoma is an electrically coupled population of neurons which produces the rhythmic bursting output which drives the motoneurons controlling the animal's feeding behavior. A major, unresolved problem in understanding the functioning of this network has been determining the origin and mechanism of burst generation. Burst generation can be expected to be either a property of individual cyberchron neurons or an emergent network property, two distinctly different mechanisms. Detailed analysis of the membrane properties of the cyberchrons has been hampered due to their small size, permitting only single microelectrode penetration. The single electrode voltage clamp (developed by Wilson and Goldner, 1975; J. Neurobiol. 6:411) is an essential technique enabling the membrane properties of such small, but important neurons to be examined.

Cyberchron neurons were specifically examined for the presence of a persistent inward current and associated negative resistance region which has been shown to underlie bursting in single molluscan neurons by other investigators. Steady-state I-V curves were found to be remarkably linear (small degree of delayed rectification) over the membrane potential range of -80 to -10 mV. The effects of loading on a cyberchron neuron by the rest of the electrically coupled network was constant for the range of command potentials employed and was calculated to be comparable to the loading of an axon on a soma of a non-electrically coupled neuron. It is concluded that cyberchrons do not individually have the membrane properties which have been considered to be essential for burst generation by single neurons, and therefore burst generation in the cyberchron network must rely on network interactions or the properties of some uncharacterized neuron.

Investigations are currently in progress to study the membrane properties of a newly identified but uncharacterized cyberchron neuron which appears to be the origin of a shunting process. This unusual cyberchron neuron may be a key element which regulates the effective coupling between the main population of cyberchron neurons by causing a shunt during the burst. A decrease in coupling between the main population of cyberchron neurons is in fact observed during and immediately after a burst which may be responsible for burst termination.

1226 RECRUITMENT OF MOTORNEURONS IN THE COCKROACH. D. Meyer* and P. Walcott. Anat. Sci., Health Sciences Center, SUNY at Stony Brook, Stony Brook, NY 11794.

We have been able to account for the recruitment sequence of a small population of insect motorneurons by their different innate sensitivities to depolarizing current. The sensitivity of a small number of motorneurons which innervate the flexor muscles of the coxal leg segment was studied by impaling processes of these cells in the neuropile and noting the mean firing frequency of the neurons during pulses of applied current. By plotting the mean firing frequencies as a function of magnitude of the stimulating current pulse, we were able to obtain f-I relations for a number of motorneurons. The motorneurons were identified by the amplitude of the spike height recorded extracellularly from the nerve to the flexor muscle and by measuring the tension developed by the motor unit when its motorneuron was stimulated with an intracellular current pulse. In all cases we found that the threshold for the initiation of repetitive firing and the slope of the f-I relation were strongly correlated with the mechanical properties of the motor unit. The more powerful units were less sensitive to depolarizing current. Thus the most powerful motor units having the highest threshold and smallest slope for the linear portion of the f-I relation. It has been shown that several of the flexor motorneurons could be driven by depolarizing a single interneuron (Interneuron I, Pearson and Fourtner, J. Neurophysiol. 38, 1975). When we compared the f-I plots for the flexor motorneurons which were obtained by stimulating interneuron I with those obtained by stimulating the motorneurons directly, we found that the recruitment order was the same as that predicted by the intrinsic sensitivities of the motorneurons to injected current. Furthermore, the ratios of the slopes of the f-I relations were similar whether the cells were stimulated directly or via interneuron I. These results strongly suggest that some innate property (or properties) of the the motorneurons determines their order of recruitment and also their firing frequences during behavior.

Supported by NIH Grant AM 18750

1227 INPUTS TO THE LOBSTER STOMATOGASTRIC GANGLION UNMASK BURSTING PROPERTIES IN MANY OF ITS MOTORNEURONS. David F. Russell and Daniel K. Hartline, Biology Dept., UCSD, La Jolla, CA 92093

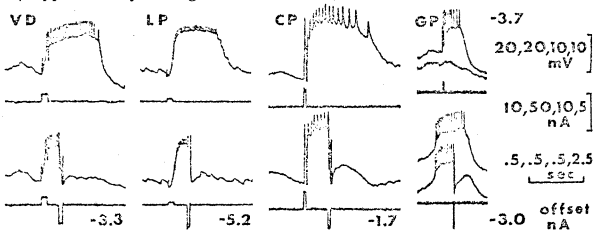
Most of the motorneurons of the stomatogastric ganglion, including the AM, CP(DG), LC(LG), and GP(MG) gastric neurons and the LP, VD, IC, early PY, and late PY pyloric-follower neurons, show prolonged regenerative responses resembling the plateau potentials in vertebrate heart muscle. This mechanism appears to make a major contribution to both gastric and pyloric rhythms.

With CNS connections intact or following stimulation of input nerves, so that the gastric and pyloric rhythms were brisk, responses could be triggered either spontaneously or with brief weak depolarizing currents (e.g. 20-50 msec, 1-5 nA; see upper figures) and terminated spontaneously or by an IPSP barrage or with brief weak hyperpolarizing currents (lower figures), typical properties for plateau potentials. They were most easily demonstrated under hyperpolarizing offsets sufficient to stop on-going firing. Plateau potentials were usually eliminated when the input nerve (Stn) was cut or reversibly blocked with isotonic sucrose. They reappeared when the block was removed or the input nerve stimulated. We hypothesize that specific transmitter substances unmask or enhance a usually latent ability of these cells to produce plateau potentials, and possibly endogenous bursting.

Plateaus increase the gain of a neuron, so that a minimum of synaptic input switches it rapidly between a strongly firing "on" state and an "off" state, allowing rapid build-up and decay of strong output effects, e.g. on muscle tension. Bursting in many networks has been considered an emergent property of synaptic interactions among neurons that have simple repetitive-firing kinetics. Alternatively, synaptic networks could be driven by plateaus acting as a motive force; e.g. plateaus in the CP, LC, and GP clearly contribute to driving the gastric rhythm.

Endogenous properties such as plateaus could play roles in other bursting networks; they may easily be overlooked unless specific tests are applied under the right conditions.

(Supported by NIH grant NS 13138.)



1228 A CRITERION OF NEURONAL SPIKE TRAIN STATIONARITY BASED ON THE TEMPORAL PATTERN OF SPIKES. Arthur C. Sanderson*, Wlodzimierz M. Kozak, Jakub Segen*, and Dan Schweitzer-Tong*. Biomed. Eng. Prog. and E.E. Dept., Carnegie-Mellon Univ., Pittsburgh, Pa.

Most methods for the quantitative analysis of neuronal spike trains require that statistical properties of the spike train do not change spontaneously with time. While the mean rate of the process is a useful indication of stationarity, it is insufficient as a statistical criterion when higher order properties are being estimated. A method has been developed based on the sequential interval histogram (SQIH) technique[1] which distinguishes between mean rate stationarity and stationarity of the temporal pattern of interspike intervals. The method utilizes a peak tracking procedure applied to the mean rate normalized SQIH data. Peak location and statistical peak significance are used as features to describe the non-stationary behavior. A statistical criterion for the stationarity of the ensemble of peak parameters has been developed. The method has been applied to neuronal spike trains recorded from single neurons of the cat brain. Examples of cells in which the pattern of spike activity changes with time while the mean rate remains constant emphasize the importance of such a strict stationarity criterion for certain classes of experiments.

[1]Sanderson, A.C. and Kobler, B. (1976) Sequential interval histogram analysis of non-stationary neuronal spike trains. Biol. Cybernetics, 22:61-71.

This work was supported in part by NSF Grant ENG-75-15736. D.S-T. was supported by NIH Research Fellowship 1-F32-EY05062-01.

1229 SENSORY MODULATION OF A CENTRALLY PROGRAMMED BEHAVIOR. Karen A. Sigvardt* and Brian Mulloney. Dept. Zoology, Univ. California, Davis, CA 95616.

The neurons of the stomatogastric ganglion in the lobster, Panulirus interruptus, generate two motor patterns which control rhythmic movements of two parts of the stomach - the gastric mill and the pyloric region. The activity of the neurons which produce the gastric rhythm and the pyloric rhythm have been described and the connections of these neurons within the ganglion are well-defined. Sensory neurons in the pyloric region of the stomach alter the activity of both gastric and pyloric neurons. Specifically, the pyloric neurons, PD and LP, are inhibited; another pyloric neuron, VD, is excited; and the gastric neurons, GM and LPGN, are inhibited. These effects correlate with discrete postsynaptic potentials in these neurons. These PSPs are accompanied by bursts in the SGN and SON nerves, nerves which connect the stomatogastric ganglion to the commissural ganglia. This sensory input, then, modulates and can reset both the gastric and pyloric rhythms. Supported by USPHS Grant No. NS-12295 and the Alfred P. Sloan Foundation. Karen Sigvardt is a USPHS-NIH Postdoctoral Fellow.

1230 EFFECTS OF MECHANICAL LOADS ON EXPIRATORY NEURONS IN THE PRESENCE OF PUTATIVE TRANSMITTERS. R. Toleikis, M. Hanley* and D. T. Frazier, Dept. of Physiology and Biophysics, Univ. of Ky., Lex., Ky. 40506

Thirty phasic expiratory neurons in the area of the Nucleus retroambigualis were studied in anesthetized cats to determine the responsiveness of this functionally homogeneous population of neurons to the iontrophorectic application of the putative neurotransmitters, glutamate and GABA. Mechanical loading of expiration, both resistive and elastic, was employed to test whether the presence of these transmitter substances altered the sensitivity of the expiratory cell to known sensory inputs. Preliminary studies with glutamate and GABA have revealed that these two substances were very effective in modulating the spontaneous activity of phasic medullary respiratory neurons.

The expiratory unit activity was analyzed with respect to: spikes/burst, burst duration, interburst intervals and average firing rate. As anticipated, addition of mechanical loads on expiration caused consistent increases in all three parameters in every cell used in the study. The firing profiles (histograms) obtained for elastic loading was quite different than for resistive loading reflecting the difference in which volume information is developed and transmitted to the brainstem. Iontophorectically applied glutamate ($\bar{x}$ = 65 nA) also resulted in modest increases in all the parameters. However, it caused little change in the overall firing profile. When the loads were applied in the presence of a sustained level of glutamate the effects were additive. The general shape of the firing profile observed with loading remained essentially unchanged. Application of GABA ($\bar{x}$ = 40 nA) resulted in a significant decrease in the parameters monitored. However, as long as phasic activity remained, loads applied in the presence of GABA produced approximately the same absolute change as they did during control. Some cells exposed to high concentrations of GABA lost their phasic activity.

This study suggests that either the synaptically activated receptors are not effected by glutamate or that these particular sites are not accessible via iontophorectic application. GABA, in modest concentrations, depressed the activity of the cells in a graded fashion, but for the most part did not interfere with the overall effectiveness of the vagally mediated input.
(Supported by NIH Grant HL 16878-03).

1231 SYNCHRONOUS BURST FIRING MODES AMONG A GROUP OF COUPLED CELL MODELS. H. Wachtel Dept. of Physiology and Biomedical Engineering, Duke University, Durham, North Carolina 27706.

From our previous voltage clamp analysis of slow wave generation in Aplysia burst firing neurons, (BFN) we have derived electronic circuit models that we are now using to study the mechanics of burst synchronization among a population of coupled cells. The synaptic interaction (chemical or electrical) between pairs of BFN models is simulated using variable gain positive feedback. Several modes of achieving synchronized slow wave (and burst) activity have been noted. The simplest of these, (and the least physiological) is based on having only one cell spontaneously oscillate, and (due to tight coupling) impose its rhythm on all other cells. A more realistic case involves coupling several cells, each of which spontaneously oscillates at a somewhat different frequency, and having them phase lock together. The most interesting mode however, results from intercoupling several "sub regenerative" BFN models, none of which oscillate on their own, but which then oscillate in concert. This last mode, which involves both "endogenous" and "network emergent" behavior, is reminiscent of synchronous burst formation in various systems, including smooth muscle and epileptic foci. In these cases, the existence of oscillatory activity also depends on the number of coupled cells involved as well as their individual characteristics. The models can also be used to demonstrate how an entire population of such cells can be triggered to switch from a "stable" (not oscillating) mode to one of sustained, synchronous, bursting upon the application of a barely perceptible stimulus.

Supported by N.I.H. Grant NS-08476.

1232 PRODUCTION OF SWIMMING BEHAVIOR IN THE MEDICINAL LEECH BY STIMULATION OF A SINGLE NEURON. Janis C. Weeks* and William B. Kristan, Jr. Dept. of Biology, Univ. Ca. San Diego, La Jolla, Ca. 92093

The medicinal leech swims by undulating its flattened body dorso-ventrally, and this behavior can be recorded in the segmental nerves as alternating bursts from the dorsal and ventral longitudinal body wall motorneurons. In response to nerve stimulation, the isolated, brainless nerve cord (a chain of the 21 nearly-identical segmental ganglia and the connectives joining them) can generate this pattern quite accurately. The central oscillator consists of a network of oscillatory interneurons in each ganglion which drives the motorneurons in the proper phase relationships and also coordinates the rhythm intersegmentally.

A neuron has been discovered which, when stimulated, causes initiation and maintenance of swimming episodes in the entire isolated, brainless nerve cord. This neuron, assigned cell number 204, is an unpaired, multisegmental interneuron which occurs in most, if not all, of the segmental ganglia. During a swimming episode, the normally silent cell 204 population is depolarized and produces a burst of impulses during each ventral contraction phase. Hyperpolarization sufficient to suppress impulse activity in any two cell 204s in the cord does not affect the generation of swimming, indicating that while any one cell 204 is sufficient to initiate swimming, the activity of at least any two is unnecessary. Stimulation of any cell 204 during an ongoing swimming episode increases the cycle frequency of the entire cord for the duration of the stimulus. Using this cell as a driver, it has been possible to produce swimming in shortened lengths of nerve cord only 3 - 4 ganglia long.

While cell 204 is evidently strongly involved with the central oscillator, it is not functionally homologous to the identified members of the oscillator, as they do not possess the "command" ability of cell 204. Determining the connectivity of this neuron to other identified neurons in the leech (sensory, oscillatory and motor) should establish its role in the natural swimming behavior of the animal, and lead to new insight into how nervous systems provide for the generation, release, and modulation of centrally patterned activities.

1233 RETINOTECTAL PROJECTION AFTER TRANSLOCATION OF TWO TECTAL GRAFTS WITHIN THE SAME OPTIC TECTUM IN GOLDFISH. Myong G. Yoon. Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada.

The topographic pattern of re-established retinotectal projection following various surgical manipulations of the optic tectum was studied in adult goldfish with neurophysiological mapping methods. In the first group of experimental fish, a rectangular piece of the tectal tissue was dissected out from the rostral zone, and a similar tectal piece was also dissected from the caudal zone of the same tectum. The two pieces of tectal grafts were rotated by 180° around the dorsoventral axis, and then the caudal graft was reimplanted into the rostral zone and the rostral graft into the caudal zone within the same tectum. A majority of the operated fish showed that the translocated tectal grafts (particularly, the rostral graft reimplanted into the caudal tectum) had undergone a complete or severe degeneration. In a few cases, however, it was possible to record visual responses from the surviving tectal grafts as well as from the surrounding intact area of the tectum. The re-established visual projections onto the reciprocally translocated tectal grafts showed the corresponding localized transposition along the rostrocaudal axis in addition to the 180° rotation around the dorsoventral axis of the operated tectum.

In the second group of experimental fish, a rectangular piece of the tectal tissue was dissected out from the medial zone, rotated 180°, and then reciprocally translocated with a similar 180° rotated tectal graft, dissected from the lateral zone within the same tectum. In the latter case, the re-established visual projections onto the operated tectum showed the corresponding localized transposition along the mediolateral axis as well as the 180° rotation around the dorsoventral axis of the operated tectum.

(Supported by grants from MRC and NRC of Canada.)

1234 MODULATION OF INPUT IMPEDANCE THROUGH RESISTIVE COUPLING.

Birgit Zipser. Dept. of Physiol., State University of New York, Downstate Medical Center, Brooklyn, N.Y. 11203.

Two classes of paired motoneurons in ganglion 6 of the leech, PS1 and PS2, that innervate the penis sheath, differ dramatically in their membrane properties, which results in a novel kind of resistive interaction. All four neurons are electronically coupled to each other. Coupling between the two PS1 is the strongest (20 to 60%) enabling them to drive each other often spike for spike as an electrical unit. The two PS2 are weakly coupled to each other (a couple of %). Coupling from PS2 to PS1 is also small.

The novel resistive interaction is brought about by current flowing from PS1 to PS2. Depolarizing current injected into PS1 elicits a significantly larger coupling potential in PS2 than hyperpolarizing current (10 compared to 4%), suggesting rectification in the coupling process. But in the case of these neurons, the asymmetry in coupling potentials does not seem to arise from the rectifying properties of junctional membrane but rather from an anomalous rectification of PS2's cell membrane.

PS2's input impedance is strongly dependent on its membrane potential. As the cell body is depolarized towards threshold, the input impedance increases tenfold. PS1's input impedance, by contrast, is constant in the vicinity of the resting potential and decreases slightly rather than increases with depolarization.

Thus, it appears that large areas of neuronal membrane of PS2 act as the rectifier. This idea is supported by the finding that PS1 can indeed modulate the input impedance of PS2. Hyperpolarizing PS1 lowers PS2's input impedance and depolarizing PS1 increases it.

Even though the coupling potentials are asymmetrical in their amplitude, they could be equivalent in inhibitory and excitatory function because of their effect on PS2's input impedance. The smaller hyperpolarizing coupling potential is significant in that it signals a decrease in PS2's input impedance. Thus, more excitatory synaptic current from other sources would be needed to bring PS2 to firing threshold.

It has been observed in several instances now in the leech that neurons within a network subserving the same function are electronically coupled. The fact that the four motoneurons to the male organ are resistively coupled is a further example. Thus, there is the possibility of delineating a functionally coherent network by using techniques that elucidate resistive junctions. (Supported by NS0577303)

NEURONAL SHAPE AND FUNCTION

1235 X-RAY MICROANALYSIS IN NEUROCHEMISTRY: LOCALIZATION OF AN *ELEMENTAL NEUROTOXIN AND MANIPULATIONS OF MITOCHONDRIAL CA++*
H.S. Adler*, J.L. Costa* and E.K. Silbergeld, NIH, Bethesda, MD 20014

The coupling of x-ray elemental analysis to electron microscopy provides a new method for studying subcellular location of elements (Somlyo et al., J. Cell Biol. (1974) 61, 723). Two problems suitable to this approach are (1) localization of elemental neurotoxins, such as Pb, whose site of action is unknown; and (2) intracellular ion changes after drug treatment. For these studies synaptosomes were purified from rat caudate, air-dried on grids and examined in the scanning-transmission mode (STEM) on a Hitachi 500 EM equipped with energy dispersive x-ray spectrometry.

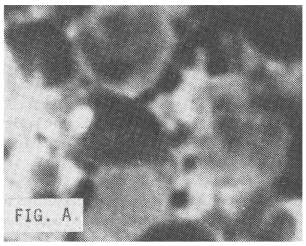


FIG. A

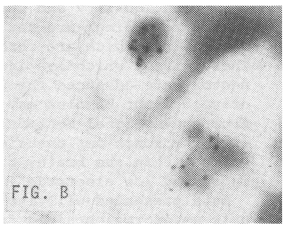


FIG. B

Air-dried synaptosomes (Fig. A, 25 KeV, 20,000x) appear as indistinct rounded shapes. Fig. B shows more lucent synaptosomal profiles at 75 KeV, 50,000x), which contain darker ovoid structures. The ovoid structures contain dense granules, which give x-ray spectra indicating Ca and P. Microprobing of the more lucent area, in contrast, gives spectra showing Na, Cl, K and P. Composition, location, and size suggest that the ovoid structures are mitochondria. In synaptosomes exposed to Pb (10^{-6} M), Pb was detected only when mitochondria were probed and was always associated with Ca and P. This suggests that Pb is transported across nerve terminal membranes and is sequestered in mitochondria. In other studies, synaptosomes and isolated mitochondria were exposed to antimycin A, reserpine, ATP, the local anasethetic N,N-diethylamino-octyl 3,4,5-trimethoxybenzoate, high Ca (4.8 mM) and 0 Na media. Changes in the intensity of the x-ray spectra for Ca were detected, consistent with the predicted effects of these treatments on Ca binding. It is concluded that x-ray microprobe with STEM electron microscopy may be useful in monitoring neurochemical processes mediated by or reflected in changes in elemental composition. Also, the technique of air drying appears to permit the study of ionic events at the mitochondrial level in synaptosomes.

1236 DENDRITIC GEOMETRY AND SPIKELESS NEURONAL COMPUTATION.
William H. Calvin and *Katherine Graubard*, Departments of Neurological Surgery and of Zoology, University of Washington, Seattle, Washington 98195 USA.

When output synapses are located near input synapses on the same cell process (axoaxonic, dendrodendritic, etc.), then local PSPs might directly affect transmitter release, especially since the presynaptic voltage threshold for transmitter release can be near the resting potential (Graubard, Raper and Hartline, this volume). Calculations using the steady-state cable equations were done for a number of different neuronal geometries, beginning with the *Aplysia* neurons whose membrane and axoplasmic resistivities were earlier determined by Graubard (Brain Res., 88:325, 1975). There is often little voltage attenuation from soma to dendritic tips, but the *attenuation from tip to soma is often severe* due to the resistance mismatch at the junction from thin to thick diameter processes. Nevertheless, *a synaptic conductance change of quantal proportions produces a somatic PSP whose size varies by little more than 20% as the site of the conductance change is moved from soma to tip.* The apparent conflict between the statements in the last two sentences is due to the compensation produced by the local size of the PSP becoming considerably larger as the conductance change is placed further out the thin dendritic process. Thus input synaptic location can be relatively unimportant for somatic PSP size. However, input synaptic location is very important when one considers what voltages are developed in thin processes where output synapses may also be located: moving an input synapse from soma to distal tip could increase this voltage more than 10-fold. *To the extent that an output synapse is isolated from the central portion of the neuron by long, thin diameter processes, it will be more heavily influenced by its neighborhood input synapses than by input synapses located centrally or upon other dendrites.* Inhibitory synapses with reversal potentials at the resting potential (pure "shunts") were also investigated for their effects upon excitatory synapses; they are more effective at reducing the PSP when they are located *at, or distal to,* the excitatory synapse. In *Aplysia* abdominal ganglion neurons, R_m is so high that little current is lost through membrane leakage within the ganglion; the above properties are entirely due to dendritic tree shape. When lobster and mammalian neurons were simulated with more typical 1000-4000 ohm·cm^2 values for R_m, leakage of current was noticeable, but the geometric effects noted above still dominate. (Supported by NIH Grants NS09677 and NS04053 and Fellowship NS05060).

1237 MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES OF SENSORY INTERNEURONS IN THE CRAYFISH. Russell A. Fricke* (SPON: Donald Kennedy). Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Using a new modification of the Timm's method for intensifying the staining of whole-mounts of cells previously filled with cobalt, the intraganglionic architecture of mechanosensory interneurons in the crayfish has been studied. Dendritic trees of these cells range along a continuum from exclusively ipsilateral to exclusively contralateral, and from complex, highly branched structures to simple patterns with relatively few branches. The topographic location of branches within the ganglion is closely related to, but not the exclusive determinant of, a cell's peripheral field. On the other hand, both branching complexity and location are related to the place occupied by a neuron in the connectivity hierarchy of central sensory interneurons. Cells located far along in the connectivity hierarchy, which are believed to receive mainly electrical synaptic input, have always been found to have simple dendritic patterns, whereas lower order cells, receiving predominantly chemical synaptic input, display considerably more complex dendritic arrangements. In this regard, sensory interneurons of different modalities (mechanoreceptive and proprioceptive) appear to resemble each other more when they occupy similar hierarchical positions than do cells of the same modality, but of different order.

1238 SPIKE COMPONENTS RECORDED IN THE SOMA OF A BIFURCATING LOCUST NEURON RELATED TO NEURAL GEOMETRY. W. Heitler* and C. Goodman* (SPON: D. Bentley). Dept. Zoology, UCB, Berkeley, CA 94720

Dorsal unpaired median (DUM) neurons are unusual amongst locust neurons in 2 respects: 1) the somata carry overshooting action potentials, 2) the axons bifurcate a short distance from the somata and exit on either side of the ganglion. Recordings from the soma of the identified DUM neuron innervating the metaextensor tibiae muscle (DUMETi) reveal 4 different spike types associated with 4 spike initiation sites (SISs). These are located 1) on the axon hillock giving rise to a soma (S) spike of 70-90 mV, 2) between the axon hillock and axon branch point giving rise to a neurite (N) spike of 20-40 mV, 3) and 4) distal to the branch point on the left and right axons giving rise to axon (A) spikes of 8-15 mV.

At spike frequencies greater than 10 Hz at room temperature or 1-5 Hz at 32°C (the preferred environmental temperature of the locust) the S spike may fail in a phenomenon similar to the initial-segment/somatodendritic break of vertebrate neurons. When the S spike fails, residual A spikes, or more rarely N spikes are revealed. A spikes usually consist of 2 more-or-less separate components, A_l and A_r, which can be correlated with action potentials in the left and right axon branches by recording spikes extracellularly in the peripheral nerves on each side. Occasionally single component A spikes occur when an action potential is initiated on only one side of the branch point. Thus action potentials may occur independently in the branches of this bifurcating neuron.

We interpret these results as indicating that there is a region of passive membrane about the branch point of the axon, such that a spike initiated in one axon branch propagates electrotonically across the branch point to the SIS on the other branch where it may (or may not) initiate a second-component A spike, and also to the neurite SIS where it may initiate a N spike. The N spike propagates to a region of low safety factor at the axon hillock where it may initiate a S spike.

In most preparations the A spike components A_l and A_r are approximately equal in amplitude, and cobalt sulfide stains show the axon branches of DUMETi to be symmetrical. However, in one case A_l was considerably smaller than A_r, and staining that neuron revealed that the left axon branch underwent a major excursion into a region of neuropil not normally invaded by DUMETi, which added at least 150 um of membrane between the branch point and the presumed SIS of the left branch. The striking correlation between the geometrical and physiological asymmetry supports our interpretation of the functional anatomy of this neuron. (Supported by NIH grant NS 09404-05)

1239 COMPUTER-AIDED RECONSTRUCTION OF GOLDFISH PHOTORECEPTOR SYNAPSES FROM SERIAL ULTRATHIN SECTIONS. D. O. Lightfoot* and W. K. Stell, Jules Stein Eye Institute, UCLA Sch. Med., and M. J. Shantz* and G. D. McCann*, California Institute of Technology.

We have analyzed contacts of photoreceptors with horizontal (HC) and bipolar cells (BC) in the retina of the goldfish. Five types of BC which contact both rods and cones can be distinguished by light microscopy, and their membrane junctions with the photoreceptors can be identified by electron microscopy, in Golgi preparations. BC types A1, A2 and A3 have thick dendrites, large somata, and long axons; their dendrites make narrow-cleft (11-13 nm) junctions, and sometimes ribbon junctions, with the receptors. BC types B1 and B2 have finer dendrites, smaller somata, and shorter axons; their dendrites make wide-cleft (16 nm) non-ribbon junctions with the photoreceptors. To further characterize the interrelations of these HC and BC dendrites with the photoreceptor synaptic endings we have used a computerized image analysis system to translate data from serial electron micrographs into quantitative form. We can retrieve descriptive data in terms of dimensions, surface areas and volumes, as well as generate three-dimensional images.

Each rod spherule contains 5-10 dendrites of second-order cells, which can be identified in serial sections by ultrastructural criteria established in the Golgi-EM studies. The single rod HC dendrite is the most prominent. It branches extensively within the invagination and around the BC dendrites, and terminates lateral to the synaptic ridge. Of the BC processes, the dendrite of type A1 is the most extensive, curving and branching as well as making extensive contact with the rod. Dendrites of the other BC's are smaller, straight and unbranched, and make less contact with the rod. Since the number of rods contacting each type of BC are known from Golgi preparations§, the total area of rod junction per BC can be calculated. We have found that the total junctional area varies dramatically from one BC type to another. Typical figures are:

BC type:	A1	A2	A3	B1	B2
Rods per BC:	218	223	100	25	20
Total junctional area: (μ^2 per BC)	138.98	30.57	1.76	1.34	0.42

These data indicate that rod pathways in goldfish are multiple and highly differentiated, and suggest that nerve cells can be further classified and characterized by quantitative properties of their dendrites.

§ Ishida, Stell & Lightfoot, in preparation.

(Supported by NIH Research Grants EY 01190 and NS 03627)

1240 QUALITY AND LONG-TERM STABILITY OF NEURONAL UNIT RECORDINGS IN UNRESTRAINED CATS. D. J. McGinty and R. M. Harper. V. A. Hospital, Sepulveda, CA 91343 and University of California, Los Angeles, CA 90024.

We have reported studies of neuronal unit discharge from amygdala, hypothalamus, midbrain tegmentum, dorsal raphe nucleus and pontine FTG field in unrestrained cats. Our method has a number of significant advantages including simplicity of procedure, long-term stability of unit recordings, ease of simultaneous recording of 2 or more units, and absence of experimental limitations imposed by physical restraint of the animal. This presentation will show the details of electrode preparation, surgical preparation, recording equipment, and document characteristics, quality and stability of unit discharge.

Unit recordings are derived from 32μm insulated stainless steel wires which are chronically implanted in small bundles. These wires, which are individually soldered to a standard connector, are advanced through the brain with a mechanical microdrive. Eight bundles may be implanted in each cat. Differences from conventional methods include flexibility of microelectrode wires, chronic implantation and relatively slow advancement of wires within the brain, chronic solid closure of cranial openings, and low electrode impedance.

Data presented will include illustration of 16 consecutive unit waveforms obtained from pontine recordings in a single subject, stability of unit waveshapes over a 5 day period, stability of behavioral correlates of unit discharge over 5 days, and simultaneous discharge of pairs of units. Rate of unit discharge from waking, slow wave sleep and REM sleep in neuronal pairs will be given for 20 consecutive sleep-waking cycles. The ranking of unit discharge rates across units was preserved in each sleep cycle. Unit potential fields were estimated from plots of spike amplitude vs. electrode depth. Pontine tegmental cells had spike fields of 500μm.

1241 REGENERATION FOLLOWING NERVE REPAIR: THE PROPORTION OF PROXIMAL FIBERS CROSSING THE ANASTOMOSIS MEASURED BY THE AREA UNDER THE MONOPHASIC COMPOUND ACTION POTENTIAL IN RATS. Joseph M. Rosen,* Don L. Jewett, and Ernest N. Kaplan.* Department of Orthopaedic Surgery, University of California, San Francisco, California 94143, and Division of Plastic Surgery, Stanford University Medical Center, Stanford, California 94305.

Estimation of the results of different types of nerve repairs by histological methods is difficult because of the presence of nerve fibers which branch or fail to function. The height of the compound action potential provides equivocal results since it varies both with distance and conduction velocity changes.

Here we report that a quantitative estimation of the proportion of proximal fibers which function across the anastomotic site can be made by measuring the area under the monophasic compound action potential. We studied the saphenous nerves from 5 rats in vitro 8 months after severance and repair. Each nerve was stimulated supramaximally for myelinated fibers both proximal and distal to the anastomosis and the ratio of the action potential areas (recorded from the proximal end in volt-sec) were compared. The recording system was accurate to better than 1%.

As shown in Table I, control nerves from the opposite limb showed variations between proximal and distal areas less than 7% despite significant decrements in action potential heights due to dispersion (first and third column). Thus the method has sufficient accuracy to detect the changes observed in the sutured nerves (columns two and four). The results from the sutured nerves show that the distal fibers are but 47% to 74% of the proximal fibers present. This decrement may be the result of fibers which end in the scar or fibers which change their diameters so severely that centripetal conduction is blocked at the anastomosis.

In this method the distally stimulated fibers are a subset of the total population of proximally recorded fibers. Thus the interpretation of the ratios in Table I requires the not unreasonable assumption that the mean volt-sec of the fibers crossing the anastomosis recorded proximally does not differ significantly from the mean volt-sec of the total population of proximal fibers.

Table I: Expressed as %: The Ratio of the Volt-Sec Resulting from Distal Stimulation Divided by Volt-Sec Resulting from Proximal Stiulation.

	AREA		HEIGHTS	
	Control	Sutured	Control	Sutured
Median	94.6	60.5	74	42
Mean	95.5	59.2	75	41
Range	93.0-101.5	46.7-74.1	63-88	26-60

1242 HOW SHAPE CAN AFFECT EXCITATION AND PROPAGATION IN NEURONS. R. M. Westerfield* and J. W. Moore* (SPON: George Somjen) Dept. of Physiology, Duke University, Durham, N. C. 27710, USA.

We have examined the separate contributions of membrane characteristics and cell shapes to excitation and propagation of impulses in neurons by experiments and computer simulations.

Squid axons were used as the experimental preparation. An increase in the electrical diameter (a lower longitudinal internal resistance) was achieved by insertion of a low surface resistance axial wire. Computer simulations of the experimental system used the Hodgkin-Huxley equations to describe uniform membrane segments in a set of difference equations describing a cable.

In both experiments and simulations, action potentials in the larger diameter ("soma") region were similar to those seen in motor neurons under antidromic invasion, showing more than one inflection point or peak.

Similar to the lower threshold observed in the axon hillock of neurons, both the current and voltage threshold were lower in the normal squid axon adjacent to that region with the wire. Under certain conditions, impulse initiation occurred in this region when current was injected into the "soma".

NEUROPATHOLOGY AND NEUROIMMUNOLOGY

1243 THE EFFECT OF AMBIENT TEMPERATURE ON THE HYPERTHERMIA EVOKED BY ACUTE MECHANICAL DAMAGE TO THE HYPOTHALAMUS. Deborah Ackerman* and Thomas A. Rudy. School of Pharmacy, University of Wisconsin, Madison, WI 53706.

Fevers induced by prostaglandins (PG) or bacterial endotoxins (E) are functionally equivalent to an upward shift in the set-point for thermoregulation. It is characteristic of these regulated fevers that their magnitude is little affected by variations in ambient temperature (T_a) within the cool to warm range. However, if core temperature (T_c) is forcefully elevated by exposure to high T_a or if the anterior hypothalamic/preoptic region (AH/PO) is locally heated, the fever is inhibited. We have reported elsewhere (Rudy et al., J. Physiol., 1977, in press) that unilateral acute mechanical lesioning of the AH/PO in the rat evokes reliably an immediate, sustained hyperthermia which may prove useful as an animal model of the intractable hyperthermia sometimes produced by cerebral trauma in man. Since the hyperthermia in rat is susceptible to indomethacin inhibition, we suggested that PG synthesis and release is ultimately responsible for the effect.

To ascertain whether fevers produced by cerebral trauma (CTF) behave like those produced by PG and E, we examined the effect of variations in T_a on CTF magnitude. Using methods described elsewhere (vida infra), unanesthetized rats restrained at a T_a of 15, 26 or 32°C (n = 10/group) or 36°C (n = 3) were subjected to acute unilateral lesioning of the AH/PO while core temperature (T_c) was monitored continuously. Fever magnitude (ΔT_c) was defined as the difference between T_c at the time of lesioning (T_{co}) and the maximum T_c reached within 3 hr (T_{cm}).

Lesioning produced a hyperthermia which began immediately, reached a plateau within 3 hr and was maintained for 8-12 hr. Fever magnitude was not significantly influenced by variations in T_a between 15°C and 32°C ($\Delta T_c \pm$ SEM: 15° = 1.75 ± 0.21; 26° = 1.80 ± 0.24; 32° = 1.87 ± 0.36). However, regression analyses of T_{co} vs. T_c and T_{co} vs. T_{cm} revealed a tendency toward inhibition of ΔT_c by high T_{co} so that T_{cm} did not exceed approximately 40°C. This impression was strengthened by supplemental tests at T_a = 36°C. In these rats, T_{co} was elevated ($\overline{T_{co}}$ = 39.35°C) and ΔT_c was strongly attenuated ($\overline{\Delta T_c}$ = 0.42°C), producing a mean T_{cm} of 39.97°C.

Although these findings alone do not suffice to prove that CTF represents a functional elevation of the setpoint for thermoregulation, they are in substantial agreement with the effect of T_a and basal core temperature on fever evoked by agents known to act by this mechanism. The data are also compatible with the postulate that CTF is mediated by the release of prostaglandins.

1244 CALCIUM DEPOSITION FOLLOWING ELECTRICAL STIMULATION OF CAT CEREBRAL CORTEX. W.F.Agnew, T.G.H.Yuen*, P.F.Johnson*, R.H.Pudenz*, L.A.Bullara* and D.Jacques. Huntington Institute of Applied Medical Research, Pasadena, California and Department of Neuroscience, University of Florida College of Medicine, Gainesville, Florida.

The effects of electrical stimulation of the cat cerebral cortex have been evaluated by light and electron microscopy following a wide variety of stimulation parameters. Calcium deposition was prominent among numerous degenerative changes in subpial tissue following stimulation with some of the more noxious parameters, e.g. 1.1 mm disc electrode, 300 ma/cm^2, 50 pps, 120 ^{2}C/cm^2/phase of biphasic-capacitively coupled stimulus. At the light microscope level calcium deposits were detected by Kossa's Alizarin stain which increased in density with the intensity of the stimulus. With the electron microscope calcium deposits appeared as dense crystalline inclusions in several cell types and varied in size from 0.1 to 3.2 μm. These intracellular inclusions were identified as crystalline calcium hydroxyapatite by electron diffraction and the presence of both calcium and phosphorus was detected in the bodies by microanalysis using scanning-transmission electron microscope with an energy dispensive spectrometer attachment. The mechanism of the calcium deposition is obscure, however, a possible explanation is that increased cyclic AMP levels result in enhanced calcium plasmalemmal permeability known to occur following electrical stimulation of the brain. Supported by NINCDS contracts NOS-NS-0-2275 and NS-1-2286.

1245 TRANSPLANTATION OF HUMAN SCHWANN CELLS TO MOUSE PERIPHERAL NERVES Albert J. Aguayo, Jack Kasarjian*, Emil Skamene*, Patricia Kongshavn* and Garth M. Bray. Depts. Neurology & Immunology, Montreal General Hospital and McGill University, Montreal, Canada.

Schwann cells can be transplanted into nerves of mice from the same or different strains (Nature 265: 73, 1977). The present communication describes the successful transplantation of human Schwann cells into nerves of immunologically suppressed mice.

Human sural nerves were obtained from amputations and diagnostic nerve biopsies and single fascicles, 5 mm in length, were grafted between the stumps of transected sciatic nerves of C57 BL/6J mice. To prevent rejection, recipient mice received antilymphocytic serum (ALS) twice weekly.

Serial ultrastructural studies from 10 days to 3 months after grafting showed no rejection of the human grafts and demonstrated an increasing number of regenerated myelinated and unmyelinated fibers within the graft and distal stump of the grafted nerves.

To confirm the presence of human cells within the grafted segment of regenerated mouse nerves, ALS was discontinued in a group of animals two months after grafting. These animals also received 10^8 lymphoid cells obtained from syngeneic mice hyperimmunized against human tissue. Under these conditions both fibroblasts and Schwann cells were rejected in the graft but tolerated in the proximal and distal stumps of the regenerated nerves. Within the graft Schwann cells of both myelinated and unmyelinated fibers were surrounded, penetrated and replaced by mononuclear macrophages. During the period of Schwann cell rejection, many axons remained naked while others degenerated.

These studies have documented that: (1) human Schwann cells can ensheath and myelinate mouse axons; (2) human fibroblasts form the perineurium of the regenerated grafts ; (3) rejection of these regenerated xenografts is directed against nerve sheath cells and does not primarily affect axons.

These in vivo combinations of transplanted human Schwann cells and animal axons should help investigate biologic mechanisms in normal and pathologic human nerves.

1246 TISSUE REACTIONS TO VERY LONG-TERM IMPLANTATION OF CEREBELLAR STIMULATING ELECTRODES IN MONKEYS. Thomas L. Babb, W. Jann Brown*, Henry V. Soper, George M. Strain*, Jeffrey P. Lieb and Paul H. Crandall. Brain Research Institute, UCLA, Los Angeles, CA. 90024.

Previous studies of tissue damage resulting from long-term electrical stimulation of the cortex of cats (Pudenz et al., Surg. Neurol., 1975, 4:389-400) or monkeys (Gilman et al. Arch. Neurol., 1975, 32:474-477; Brown et al., J.Neurosurg., 1977, in press) have emphasized that neural damage increases directly with increased charges delivered over long periods. However, in most of these controlled studies the stimulating and control (unstimulated) electrode arrays were implanted for several months but never as long as one year. In two monkeys whose cerebella were examined after two platinum disks (each 6.8 mm^2) had been on the pia of the cerebellum for over one year there was deep compression atrophy of the cerebellum beneath unstimulated electrodes. This compression was due to a large meningeal encapsulation of the electrode array. In one of the monkeys, the opposite cerebellar cortex had been stimulated at various intervals at charges known to produce neural damage, but the encapsulation and atrophy was less than that beneath the unstimulated electrodes on the opposite paravermal cortex. These results suggest that in certain cases very long-term implantation of large electrode arrays may result in cerebellar damage greater than if the electrodes had been stimulated.

Supported by NIH Contract NS4-2331 and NIH Grant NS12054.

1247 THE EARLY SYMPTOMS OF MULTIPLE SCLEROSIS: EFFECTS ON SUBSEQUENT NEUROPSYCHOLOGICAL TEST PERFORMANCE. Samuel D. Brinkman and Francisco I. Perez*. Neuropsych. Lab., Dept. of Neurology, Baylor Col. of Med., Houston, Tx 77025.

Sixty MS patients were administered a neuropsychological test battery which included tests of cognitive and intellectual functioning, sensorimotor functioning, and basic motor abilities. The sample was partitioned in three different ways: 1) by duration of MS symptoms (cutoff set at nine years); 2) by type of initial symptoms (motor, somesthetic, or visual); and 3) by laterality of initial symptoms (right, left, or non-lateralized). Univariate F-tests indicated significantly lower memory functioning in patients with longer duration of symptoms. F-tests between the other two sets of groups showed no clear pattern. Discriminant functions were then calculated for each type of grouping, employing the WAIS and Wechsler Memory Scale (WMS) as predictors. The discriminant functions suggested that, on a multivariate level, tests of higher cortical functioning requiring little motor ability could significantly contribute to the differentiation of groups. Additionally, the optimum functions for classification appeared to be qualitatively different when calculated for duration groups, groupings based on type of initial symptoms, and groupings based on laterality of initial symptoms.

1248 LIGHT AND ELECTRON MICROSCOPIC STUDY OF SUPPORTING CELL PHAGOCYTIC AND GLYCOLYTIC ENZYME ACTIVITY IN PERIPHERAL NEUROPATHY. Keith A. Carson, Gary H. Duncan*, Wallace A. Ambrose*, and Jacob S. Hanker, Dental Research Center and Neurobiology Program, U.N.C. Chapel Hill, North Carolina 27514

Peripheral sensory nerves of inbred mice (dt/dt) afflicted with dystonia musculorum are known to undergo spontaneous degeneration soon after birth. Mice (db/db) afflicted with an inbred disorder resembling late onset human diabetes mellitus were found to display milder, though similar, neurologic symptoms to the dystonics late in the disease. A Schwann cell hyperplasia was noted in the trigeminal ganglion (TGG) and femoral nerves of both strains of afflicted animals. Metabolic activity of cells in the TGG was assessed by two methods: (1) localization of tail vein injected horseradish peroxidase in the TGG and (2) localization of glycolytic enzymes lactate dehydrogenase (LDH) and nicotinamide adenine dinucleotide dehydrogenases (NADH-DH) by catalytic osmiophilic polymer generation. TGG from normal and afflicted littermates were compared.

TGG stained for HRP had numerous dark granules localized within Schwann cells, satellite cells, fibrocytes, leucocytes and capillary endothelial cells, as verified by electron microscopy of adjacent thin sections. The TGG from the dystonic animals had a marked increase in cells containing HRP, while the TGG of the diabetic was not significantly different from the normal in this regard. The majority of reactive cells in the TGG of the dystonic were Schwann cells and satellite cells. Cells with stained granules were less common in normal littermates and nonexistent in uninjected control animals.

LDH staining in neurons was localized to the reticular membrane system and the axolemma. Mitochondrial staining was demonstrated under appropriate fixation conditions. In the TGG stain was also found on the plasma membrane and reticular membranes of Schwann cells, satellite cells, fibrocytes, leucocytes and capillary endothelial cells. Dystonic animals showed a marked decrease in neuronal staining. However, Schwann and satellite cell staining was increased. Stained vacuoles filled with membranous material were common in Schwann cell cytoplasm. Staining for NADH-DH shows the same differences between normal and dystonic animals, but its localization is cytoplasmic rather than in association with intracellular membranes. In the diabetic strain differences in staining for both enzymes in normal and afflicted animals could not be unequivocally demonstrated.

Although in dystonic mice the Schwann cell hyperplasia in TGG is accompanied by increased phagocytic and glycolytic enzyme activity, in diabetic mice where the hyperplasia is less marked, these metabolic changes were not evident. Supported by NIH grants DE-02668, DE-00288, HL 15888, DE 07018 and RR 05333.

1249 A COMPARATIVE STUDY OF QUANTITATIVE SENSORY TESTING AND PERIPHERAL NERVE CONDUCTION VELOCITIES IN HUMAN DIABETIC SUBJECTS. J.P. Conomy, K.L. Barnes, and A.J. Wilbourn. Dept. of Neurology and Division of Research, Cleveland Clinic Foundation, Cleveland, Ohio, 44106.

Slowing of the peripheral nerve conduction velocity is widely accepted as evidence of peripheral nerve dysfunction. It is well known, however, that there are individuals who acquire peripheral nerve disease in whom the clinical electrophysiologic studies of peripheral nerve functions remain normal. We studied a group of 15 diabetic subjects by a method of quantitative sensory testing (QST) previously described by Conomy and Barnes. This method is an adaptation of the method of limits and employs electrical stimuli to study perceptual aspects of skin sensation. Eleven subjects also underwent extensive nerve conduction velocity (NCV) studies in an effort to correlate the results of the clinical examination and of QST with NCV abnormalities. Diabetic subjects included men and women with both juvenile and adult onset diabetes, and individuals receiving insulin as well as those controlled by other drugs or diet alone. All individuals in the study demonstrated clinical signs and symptoms of neuropathic disease. QST studies showed definite abnormalities in the legs of 14/15 subjects, and in both arms and legs of 7/15. No patient with diabetic neuropathy had involvement of the arms alone. One subject, a 17 year old insulin-dependent diabetic, showed no QST abnormalities. Eleven subjects had both QST and NCV testing. Both studies were abnormal and consonant with the clinical picture in 6, and partially consonant in one. In this subject, QST abnormalities were detected in the hands and feet, while NCV's were abnormal in the feet alone. Only the QST findings were abnormal in 4 diabetics. In no individual in the QST-NCV group were both studies normal, and in none was NCV alone abnormal. Our studies suggest that while NCV's may be normal in the diabetic with clinical neuropathy, in some subjects QST defines laboratory evidence for peripheral nerve dysfunction. In many subjects both types of studies are abnormal, and are complimentary in the documentation of peripheral nerve disease.

1250 NEUROLOGICAL EVALUATION OF THERAPIES FOR CEREBRAL ISCHEMIA IN THE MONGOLIAN GERBIL. Barbara A. Dodson*, Dept. of Anesthesiology University of North Carolina, Chapel Hill, NC 27514; and Charles J. Hannan, Jr., Dept. of Radiology, Medical College of Georgia, Augusta, GA 30902.

The Mongolian Gerbil is a convenient model for cerebral ischemia (stroke) due to the absence of a posterior communicating artery in many of the animals. In this study, gerbils of either sex (50-60 gms) were anesthetized with ketamine (44 mg/kg), restrained and the left common carotid artery exposed with a midline incision. The freed carotid was clamped with a Schwartz spring clip for one hour. At the end of the hour, the animal was reanesthetized, the clip was removed, and the wound sutured. Within five minutes of clip removal the animal was given an intraperitoneal injection of either saline or experimental drug. Neurological evaluation (using a stroke index similar to that of McGraw et al. Stroke 7:485-488, 1976) commenced one hour after clip removal and was performed every eight hours for four days. Using a stroke index provided quantitation of the trauma but made the categorization of animals into either a stroked or non-stroked group difficult in many cases.

None of the groups receiving experimental drugs exhibited benefits greater than the control group. Drugs used included: DMSO (2 g/kg in 50% solution), and the following drugs dissolved in 50% DMSO in a total volume of 0.25 ml/animal: pyridinol carbamate (10 mg/kg), pentoxifylline (5 mg/kg), spermine and spermidine (10 mg/kg of each). One group of animals received a pretreatment with p-chlorophenylalanine (PCPA) (400 mg/kg) which was also found to not be significantly beneficial.

		MEAN STROKE INDEX*		
GROUP	(Mortality/Total)	1 hr.	24 hr.	Day 4
Saline	(1/27)	6.6±3.1	5.4±2.7	3.2±2.2
DMSO	(1/8)	9.7±3.8	6.3±3.4	3.6±2.3
Pentoxifyllin	(0/9)	8.3±3.5	6.4±2.8	3.7±1.3
Pyridinol carbamate	(3/8)	14 ±5.6	8.6±4.5	4.3±7.8
Spermine + Spermidine	(0/9)	14 ±2.8	5.6±3.4	3.6±1.9
PCPA	(1/5)	7 ±4.5	4.4±3.6	2.3±2.2

*plus and minus standard deviation

Dimethylsulfoxide (DMSO) has been reported to be helpful in experimental stroke (de la Torre and Hill. 1975. Dynamics of Brain Edema, p. 306, Spinger-Verlag) when administered intravenously, which may explain our lack of success with intraperitoneal injection. A larger dose of DMSO may prove beneficial (we have determined the LD 50 for DMSO in the gerbil to be 22 g/kg ± 1g), however, intravenous administration could be a critical necessity even though DMSO has remarkable tissue penetrating properties.

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1251 NEW SCANNING AND IMAGING TECHNIQUES IN NEUROLOGICAL COMPUTED TOMOGRAPHY: CLINICAL ASSESSMENT. T. Blaise Fleischmann, J. M. Dooley*, H. E. Rosenbaum, Neurological Scanning Center, and Artronix Inc. St. Louis, MO. 63144.

Initially, diagnostic imaging of the brain using computerized Axial Tomography utilized very limited scan data in very limited ways. We have developed, used, and clinically assessed innovative scanning methods and presentations of scan data.

Computer programs were first of all developed to permit flexibility of scanning methods. These programs took full advantage of computerized patient positioning, low total skin dosage, 3mm thick slices, and specialized reconstruction programs available with Artronix Neuro-CAT scanner. Previous scan methodologies employed fixed procedure duration and resulted in fixed image thickness and diagnostic resolution. Neuroscan procedures which we have developed vary total scan time and patient radiation dose and result in variable image thickness and diagnostic information content. The type of scan procedure to be used is matched with patient diagnostic need at the time each patient is scheduled for examination. Scans resulting in contiguous 3,6, 9, or 12mm images can be selected. In addition, the ability to chain subprocedures permits a view of Posterior Fossa contents with 3mm images while also providing 12mm images through supratentorial structures. This capability to optimize the scanning procedure to fit patient need has led to the establishment of a variable cost structure for examinations. Application of particular scan procedures to specific cases is discussed.

Computer programs were also developed to permit flexibility of scan data presentation. For instance, clinically-useful, 3mm thick sagittal and coronal projections of intra-cranial structures are routinely obtained in seconds. These are produced from stored axial data without specialized patient positioning, rescanning, or "overlapping scanning". With growing expertise, production of coronal and sagittal images is increasingly limited to specific types of cases. However, when coronal and sagittal images are indicated, these have sometimes proven crucial in neurosurgical judgements and for exquisite localization during neurosurgery.

Other programs designed to add diagnostic flexibility to scan data presentation are discussed, including:

(1) Assessment of ventricular volume,
(2) Outlining other irregular three-dimensional intracranial areas of interest,
(3) "Region of Interest" image statistics,
(4) Linear CT density profiles in planes orthogonal to the scan plane, and
(5) Reconstructive magnification of specific areas of clinical interest.

1252 STUDY OF LDH ISOZYMES IN HUMAN BRAIN TUMORS. B. P. Garg#, M. Fredericks#, S. Hoelscher#, and R. B. Ramsey. (Spon. V. W. Fischer), Department of Neurology, St. Louis University School of Medicine, St. Louis, Mo. 63104.

We have studied the multiple LDH isozymes in 35 primary and metastatic brain tumors. It has been postulated that the pattern of LDH isozymes in various tissues is dependent upon their predominant mode of carbohydrate metabolism. It is believed that LDH 1 (H_4 form) predominates in aerobic metabolism, LDH 5 (M_4 form) in anaerobic. Availability of different types of tumors presents an opportunity to study these isozymes in the hope of finding distinctive patterns that may help in identification of each tumor type. These observations may also give some insight into tumor metabolism and aid in selection of chemotherapeutic agents.

Tumors were obtained at the time of surgery and subsequently lyophilized. LDH isozymes were separated on 5.5% polyacrylamide gels followed by staining by the standard procedures. Isozyme distribution was determined by densitometry. H/M ratios were calculated for the purpose of comparison. Preliminary experiments indicated that there was no major difference in the isozyme pattern of fresh versus lyophilized tissue.

TABLE I

Tissue	H/M Ratio Mean ± S.E.	N	p Value
Control	4.75 ± 0.55	4	
Metastatic	2.01 ± 0.44	4	<0.001
Schwannoma	2.35 ± 0.18	3	<0.02
Meningioma	3.01 ± 0.47	7	<0.05
Low Grade Astrocytoma	3.25 ± 1.11	3	>0.2
Glioblastoma	4.08 ± 0.60	4	<0.2
Malignant Astrocytoma	19.7 ± 3.62	3	<0.01
Melanoma	2.76	2	
Oligodendroglioma	6.09	1	
Chromophobic adenoma	2.81	1	
Craniopharyngioma	1.94	1	

Our control value is close to that found by others previously. Metastatic tumors had the lowest H/M values. Schwannoma, meningioma and low grade astrocytoma also had H/M ratios lower than control. Malignant astrocytomas had higher H/M values. It was found that isozyme patterns (zymograms) were quite distinctive for tumor types. These will be shown.

1253 CEREBELLAR DEVELOPMENT DURING GRAFT-VERSUS-HOST DISEASE. W. Sue T. Griffin, Judith R. Head*, and Donald J. Woodward. Dept. Cell Biol., U. Tx. Health Science Center at Dallas, Dallas, TX 75235.

Graft-versus-host disease (GVHD) is a debilitating and often fatal immunological condition induced by immunocompetent lymphoid cells "grafted" either experimentally or naturally into a foreign host which is immunologically incompetent. In laboratory rodents, GVHD can be procured by a variety of means, including injection of neonatal animals with allogeneic lymphocytes prior to maturation of immune responsiveness. Although numerous investigators have extensively described GVHD-induced alterations and lesions in various tissues, the central nervous system has been generally overlooked in studies of GVHD. The purpose of this study was to investigate whether the immunologically-mediated alterations characterizing this syndrome are accompanied by a decrease in the rate of cell proliferation in the neurogenesis of the cerebellum. We have shown that the developing cerebellum is severely affected during acute, neonatally-induced GVHD in F_1 hybrid rats. On the day of birth, (Fischer x DA)F_1 neonates were injected in the facial vein with 30×10^6 parental strain lymph node cells, a dose known to cause fatal wasting in virtually all animals. Uninjected neonates and neonates injected with syngeneic cells served as control animals. The affected animals, when compared with both control groups, showed a significant decrease in their ability to incorporate ^{3}H-thymidine into the cerebellar tissue DNA fraction on day 12. A single injection of ^{3}H-thymidine (2 µCi/gbw) was given 2 hrs before analysis. A decreased ability to synthesize DNA was also seen at postnatal days 13 and 14. By day 13, the mean DNA content per cerebellum was decreased, but not significantly so, while both RNA and protein contents per cerebellum were decreased significantly. On day 14, the DNA, RNA, and protein contents per cerebellum were significantly decreased as was the amount of ^{3}H-thymidine incorporated into the tissue DNA fraction. Treated animals were sacrificed when splenomegaly confirmed GVHD, but before whole body wasting was evident. Therefore, it appears that factors other than nutrient availability are mediating changes in cell acquisition and composition during early GVHD in developing cerebellum. These results describe a hitherto unrecognized phenomenon which could lead to deficits in brain neurogenesis as a consequence of clinically-induced GVHD during early childhood or fetal and/or neonatal acquisition of maternal lymphocytes.

1254 EARLY INTELLECTUAL DEFICIT IN FERRETS WITH CHEMICALLY INDUCED LISSENCEPHALY. R. Haddad, A. Rabe, J. Shek, R. Dumas*, and K. Wisniewski*. Neuroteratology Laboratory. Institute for Basic Research in Mental Retardation. Staten Island, NY 10314.

We have previously reported (Neuroscience Abstracts, 1976, 2, #1055) that adult ferrets with lissencephaly have a marked intellectual deficit. Lissencephaly was produced by exposing ferrets to methylazoxymethanol acetate during fetal development (i.e., a single dose of 15 mg/kg was injected in the pregnant ferret on gestation day 32). In addition to lissencephaly, all had pronounced hydrocephaly. Although hydrocephaly was evident in animals sacrificed at 6 weeks, it was more pronounced in animals sacrificed at 19 weeks, and still more severe in those sacrificed after behavioral testing at one year. These observations led us to question whether the intellectual deficit observed in the adult was primarily a consequence of a progressive hydrocephaly. We therefore prepared new animals to determine whether intellectual deficit could also be detected in young lissencephalic ferrets. These animals were tested in the Lashley III maze at the age of three months. The young lissencephalic ferrets (n=11) were significantly ($p<.002$) inferior to their normal mates (n=13) in learning the Lashley III maze and were as impaired as the adult lissencephalic group (n=7) had been on this test. As expected from previous observations (Developmental Psychobiology, 1976, 9, 311-314), the young normal group did not differ from the normal adult group (n=7).

To further analyze the nature and development of the brain lesions in the lissencephalic ferret, we studied the acute lesions in the fetus on 5 consecutive days after treatment as well as the first 6 weeks of postnatal brain development. In the fetus there were extensive necrotic foci in the developing cerebrum and in the posterior region of the dorsal mesencephalon. In contrast, no extensive foci of necrosis were found in the developing cerebellum although at birth and during early postnatal period the cerebellum of the lissencephalic ferret was much smaller and less differentiated than normal. The persistence of the external granular layer past 6 weeks in the lissencephalic, but not normal, ferret thus reflected retarded development of the cerebellum. Although ectopias could be found in the cerebellum of lissencephalic ferrets at weaning, the cerebellum approximated normal structure and size and did not show the extensive lesions seen in ferrets similarly treated in the perinatal period.

Hydrocephaly as well as lissencephaly were clearly evident before two weeks of age.

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1255 ALTERED CEREBROSPINAL FLUID (CSF) COMPOSITION IN EXPERIMENTAL IMMUNE COMPLEX DISEASE. A.A. Hoffman*, R.J. Harbeck*, S.A. Hoffman*, and D.W. Shucard. Dept. Medicine and Dept. Behavioral Sciences, Natl. Jewish Hosp. and Res. Ctr., Denver, CO 80206.

It has been suggested that immune complex deposition in the choroid plexus of patients with systemic lupus erythematosus (SLE) may result in alterations in CSF constituents and possibly lead to the variable neuropsychiatric disorders observed in these patients. To assess this possibility acute immune complex disease was induced in New Zealand white rabbits by a single, large dose of bovine serum albumin (BSA). Control animals received only saline. All animals were sacrificed fourteen days later and the kidney, choroid plexus, serum and CSF obtained for analyses. Using a fluorescent antibody technique, animals of the experimental group displayed unequivocal evidence of immune complex deposits in the choroid plexus, whereas none of the controls displayed such evidence. Several animals of the experimental group also showed elevated CSF protein as compared to the control group. The concentration of albumin and immunoglobulin G (IgG) in the CSF was assessed by an electroimmunodiffusion technique. In the majority of the animals showing elevated CSF protein there was a concommitant rise in both CSF albumin and IgG. In addition, some animals displayed an elevation in their CSF IgG : albumin ratio as compared to serum values. The CSF glucose level, expressed as a percent of the serum level, was in several instances below that observed in the control animals. Furthermore, antibody to BSA was found to be elevated in the CSF of a few animals.

These results indicate that immune complex deposition in the choroid plexus, a structure in part responsible for the composition of CSF, may result in functional damage to this tissue. These results are comparable to those observed in the CSF of SLE patients with CNS involvement. This model may be useful for studying the role of immune complexes in mediating CNS abnormalities in SLE. (Supported in part by USPHS NS-12394.)

1256 A RELIABLE STEREOTAXIC TECHNIQUE BASED ON AN INTRACEREBRAL COORDINATE SYSTEM IN THE CAT. I. Ilinsky*, K. Kultas-Ilinsky, and K. R. Smith. Depts. Anat. and Surg., St. Louis Univ. Sch. Med., St. Louis, MO 63104.

A stereotaxic technique using intracerebral landmarks permits much more precision in the placement of electrodes or injection needles in discrete brain loci as compared to the classical Horsley-Clarke system based on extracranial coordinates. This technique was developed in cats whose weights ranged from 2.1 to 6.0 kg. In spite of large variations in the head dimensions it was observed that the distance between the anterior (CA) and posterior (CP) commissures was extremely constant (mean 8.1 mm; standard deviation 0.31). Thus the CA-CP line was chosen as a basic reference line for the intracerebral coordinate system and an atlas of the cat brain in this coordinate system was made. The atlas was prepared using serial histological sections cut in the transverse plane perpendicular to the CA-CP line and in the sagittal and horizontal planes parallel to the CA-CP line. The entire stereotaxic procedure was monitored by contrast ventricular radiographs. The first step involved visualization of the CA and CP in a sagittal radiograph and visualization of the midline in a transverse radiograph. The coordinates for the selected target were then determined from the atlas and transferred to the transverse and sagittal ventricular radiographs. The trajectory of the electrode or needle was monitored radiographically and corrected in each of the three stereotaxic planes. When the tip of the instrument reached the desired part of the target, a lesion or injection was made. This technique was used to place electrolytic lesions or injections of tritiated amino acids into either the anterior, intermediate, or posterior part of the substantia nigra pars reticularis in 19 cats. The technique proved to be highly accurate in that the lesions or injections were precisely confined to each of the chosen areas. In none of the animals did the lesions or injections miss the desired location. (Supported by USPHS FR 05388.)

1257 DEVELOPMENT OF A MICRO-STEREOTACTIC METHOD FOR THE LOCALIZATION AND REMOVAL OF SMALL CNS LESIONS. Deane B. Jacques, C. Hunter Shelden*, Robert E. Frazer* and Harold R. Lutes*. Huntington Institute of Applied Medical Research and Jet Propulsion Laboratories of the California Institute of Technology, Pasadena, CA 91105.

The authors describe a novel stereotactic method for the removal of CNS lesions as small as five millimeters in diameter. With the development of computerized axial tomography, such small lesions can be detected by non-invasive (x-ray) scanning with computer processing of the data and removed by a combination of apparatus and procedures described herein. Newly developed surgical instruments are mounted on a micromanipulator for guidance at the operative site. These include stereo endoscopes with xenon arc illumination; a tissue expander for exposing the operative area; a radiation tracer probe; a rotary extractor; an ultrasonic microdissection probe; an aneurysm clamp micromanipulator; and instruments for operating and removal of blood from a small intracerebral hemorrhage. The micromanipulator for guiding the tactical instruments is in turn mounted on a stereotaxic guide mechanism which accurately defines all areas of the cranium in three dimensional coordinates. The combination enables the site of a tumor, aneurysm, hematoma, etc., the three dimensional coordinates of which have been located by computerized axial tomograph scan, to be accurately accessed by the stereotaxic guide/micromanipulator assembly.

1258 A MORPHOLOGICAL STUDY OF NEURONAL ALTERATIONS IN THE CAT BRAIN FOLLOWING GLOBAL ISCHEMIA. Larry W. Jenkins*, John T. Povlishock*, and Donald P. Becker. (Sponsored by J.A. Astruc). Departments of Anatomy and Neurological Surgery, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia. 23298.

Physiologically controlled and monitored, normothermic, adult cats ventillated on 75% N_2O and 25% O_2, were subjected to total cerebral ischemia via the ligation of all primary cranial arteries and their collaterals. During the experimental procedures systemic arterial blood gases and acid-base balance were maintained within normal limits. The ischemic insults were produced by the clamping of the brachiocephalic artery for durations of 5, 15 and 30 minutes. Immediately upon the release of the brachiocephalic clamp, the animals were perfused transcardially with aldehydes. Tissue samples from selected cortical areas were then processed for routine electron microscopy.

In all ischemic insults, cortical neuronal alterations were observed. In insults of 5 minutes duration, ultrastructural examination demonstrated nucleolar and chromatin condensation, and aggregations of nuclear interchromatic and perichromatic RNP granules. Cytoplasmic organelle changes consisted of occasional degranulation and pairing of rough endoplasmic reticulum (RER) cisternae. After 15 minutes of ischemia comparable nuclear and nucleolar changes were seen. Also, cytoplasmic changes were seen reflected in occasional dilation of rough and smooth endoplasmic reticulum, an increased number of degranulated and paired RER cisternae, and numerous mitochondria showing enlarged intracristal spaces. After 30 minutes of ischemia, the neuronal nuclei showed increased chromatin and nucleolar condensation. A decreased number of perichromatic and interchromatic RNP granule aggregates were also observed. Cytoplasmic changes were revealed by increased cytoplasmic volume, swollen mitochondria with disrupted cristae, dispersed RER cisternae and Golgi saccules, and organelle membrane disruption. On the basis of morphology alone, this data suggests that in acute ischemia (5 to 15 min.), those subcellular structures implicated in both RNA and protein synthetic pathways are the first and most severly damaged.

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1259 ANALYSIS OF MS AND CONTROL SERA BY RADIOIODINATION AND POLY-ACRYLAMIDE GRADIENT ELECTROPHORESIS. Steven Kornguth, Timothy Johnson*, Ursula Juhl*, Carol Steinhart*, John Sever and Maneth Gravell. Dept. of Neurol. and Physiol. Chem., Univ. Wis. Med. Ctr., Madison, WI 53711 and NIH Bethesda, MD.

The sera of 4 female MS patients in exacerbation, 1 male MS patient in remission and 4 control subjects (2 male, 2 female) of similar ages were obtained. The sera were centrifuged at 30,000 g for 30 min. and that supernatant was centrifuged at 100,000 g for 1 hr. The resultant pellet was homogenized in 0.1 M phosphate buffer (pH 7.0), again centrifuged at 100,000 g and suspended in 50 µl of the same phosphate buffer. The patients in exacerbation yielded 100,000 g pellets that contained 20-30 µg protein/ml serum; the pellet from the patient in remission contained 10-15 µg/ml and that from the controls contained 5-10 µg/ml. The proteins in the 100,000 g pellets were radiolabeled with ^{125}I using lactoperoxidase and H_2O_2 as the catalytic agents. The radioiodinated proteins were reduced with β-mercaptoethanol, dissociated with 1% SDS and then analyzed by electrophoresis on 28 cm long slab gels containing a 6-20% gradient of acrylamide. For each subject identical amounts of protein (50 µg) were applied to the gels for electrophoresis. After electrophoresis the gels were stained with coomassie blue, destained, dried and x-ray film was then placed over the gels. When compared with the control samples, all the MS patients in exacerbation had elevated levels of proteins (or subunits) with MW of 58,000; 53,000; 43,000. One of the patients in exacerbation had an elevated level of the 61,000 dalton protein. The patient in a state of remission had no changes in concentration of serum proteins or subunits. The identities of the serum proteins that are present in elevated concentrations in the MS patients have not been elucidated; it was previously shown in this laboratory, however, that the α_2 globulin, haptoglobin, is elevated in the serum of MS patients in exacerbation. Because of the possible viral etiology of MS, it is of interest that the nucleocapsid protein of measles also has a molecular weight of 60,000.

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1260 LESIONS OF THE MYOFIBRIL IN AN EXPERIMENTAL HYPOXIC MYOPATHY RELATED TO ISOMETRIC EXERCISE. Ralph W. Kuncl. Depts. Pathology and Psychiatry, U. Chicago Pritzker Sch. Med., Chicago, IL 60637.

In non-necrotic muscle fibers the pathogenesis of the commonly occurring, nonspecific myofibrillar lesions--patchy areas of (1) disruption of normal myofibrillar banding and (2) smearing of the dense Z-band--is obscure. According to Engel (*Med Clin North Amer* 52: 909, 1968) and others, such lesions occur selectively in red muscle (are perhaps specific to soleus), and they are somehow related to mitochondria, since such lesions were not seen without decreased mitochondria but decreased mitochondria occurred without myofibrillar degeneration. I studied the development in seven different muscles of an experimental myopathy in which myofibrillar lesions are essentially the only significant pathologic change. The myopathy is produced by restraining rats after pretreating them with a psychomotor stimulant, and its extent is related to the amount of isometric exercise performed by the constantly struggling rat.

The predominant lesion, occurring in 50-60% of fibers, was extensive myofibrillar disruption, which was temporally related to the 30-fold increased plasma CPK activity that began within minutes of treatment. Focal mitochondrial loss occurred subsequent to mitochondrial degeneration within areas of myofibrillar disruption. Initially, myofibrillar disruptions contained disoriented, clumped, abnormally staining mitochondria with concentrically packed cristae. Within 24 hr after onset of myofibrillar disruption mitochondria typically were lost in disrupted foci. This correlated well with a 40% reduction in specific activity of mitochondrial succinate cytochrome C reductase (SCR) early-on after treatment and a recovery of normal specific SCR activity at 24 hr. Significant Z-band smearing occurred independently of myofibrillar disruption, being noted first at 24 hr after treatment.

Both myofibrillar lesions also occur in normal untreated control muscles. In control muscles of the lower limb Z-band smearing occurred predominantly in mitochondrial-rich (red) fibers; to the contrary, myofibrillar disruption occurred predominantly in mitochondrial-poor (white) fibers. In both experimental and untreated muscle, Z-band smearing occurred predominantly in those specially differentiated peripheral areas in the muscle fiber which are immediately adjacent to transversely coursing blood vessels. These areas normally lack mitochondria.

Thus, the focal mitochondrial *loss* that occurs in areas of myofibrillar disruption in white muscle is not of the same significance as the focal mitochondrial *absence* in areas of Z-band smearing in red muscle. In the former lesion, focal mitochondrial degradation is an early concomitant and focal mitochondrial loss a subsequent event to the myofibrillar lesion; in the latter lesion focal mitochondrial absence provides a structural substratum for the occurrence of a Z-band abnormality.

1261 ADRENAL MEDULLA SPECIFIC AND BRAIN SPECIFIC ANTIGENS DETECTED BY ANTISERA TO A RAT PHEOCHROMOCYTOMA CLONAL CELL LINE. Virginia Lee*, Lloyd A. Greene*and Michael L. Shelanski*. (Sponsored by A.V. Lorenzo). Dept. of Neuropathology, Harvard Medical School and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, MA. 02115.

A rat pheochromocytoma clonal cell line, PC12, has been established and was shown to display well-defined reversible differentiated properties in response to nerve growth factor (NGF). In the absence of NGF, PC12 cells morphologically resemble mature adrenal chromaffin cells and are capable of releasing, synthesizing and storing catecholamines. After several days of treatment with NGF, PC12 cells acquire properties of sympathetic neurons in that they extend long, branching neurites and become electrically excitable. In this study antisera were prepared from guinea pigs using PC12 cells grown in the presence and absence of NGF as antigens. After extensive absorption with rat liver, kidney, spleen and thymus, these antisera retain high titers as measured by microcomplement fixation assay against PC12 cells (both NGF treated and untreated) adrenal medulla, brain and superior cervical ganglia but not against liver, kidney, spleen and thymus. Cross-absorption of the antisera with brain and adrenal medulla showed the presence of adrenal medulla specific, brain specific and PC12 cell specific antigens. Indirect immunofluorescence studies with the antisera detect antigens on both cell bodies and neurites of living PC12 cells. This indicates that at least some of the antigens are on the cell surface. Similar results were obtained with antisera which were absorbed with either brain or adrenal medulla or with both. Staining was also seen with both brain-absorbed and unabsorbed antisera in sections of the adrenal medulla but not of the adrenal cortex. Localization in brain with adrenal-absorbed or unabsorbed antisera showed diffuse staining in all areas of the brain. Adrenal medulla and brain specific antigens were not species specific since they are also found in the adrenal medulla and brain of rabbit, mouse and cat as well as rat. Among clonal cell lines tested (C_6 glioma, C1300 neuroblastoma, 3T3 & L-cell fibroblasts etc) only PC12 and clones of neural origin were recognized in the microcomplement fixation and ^{51}Cr release cytotoxicity assays by the antisera. These results indicate that PC12 cells and antisera directed against them may be used to identify specific cell markers of adrenal medullary and brain cells.

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1262 THY-1—A THYMUS ANTIGEN ON RAT NEURONES AND GLIA. Jayne F. Lesley* and Vanda A. Lennon. Molecular Neuropathology Laboratory, Salk Institute, San Diego, CA 92112.

Thy-1 (θ) is a cell surface differentiation antigen expressed in developing and adult brain and on thymocytes and peripheral T-lymphocytes of rats and mice. In order to establish with which cells Thy-1 might be associated in the central nervous system (CNS), we examined serologically cell lines derived from rat CNS (Schubert et al., Nature 249: 224, 1974). CNS cells were tested with rabbit anti-rat thymocyte serum (ATS) for their capacity to absorb complement-dependent cytotoxicity of ATS for a ^{51}Cr-labelled Thy-1 positive lymphoma line. The cells were tested also by indirect immunofluorescence with sequential addition of ATS and fluoresceinated goat IgG anti-rabbit IgG. Thy-1 specificity of ATS was established by precipitation from mouse thymocytes of the ^{125}I-labelled 25,000 dalton glycoprotein bearing Thy-1 alloantigen (Trowbridge et al., Nature 256: 652, 1975) and by absorption of both cytotoxicity and immunofluorescence activities with whole rat brain or rat thymus. One of 6 neuronal lines tested and 8 of 15 non-neuronal lines absorbed anti-Thy-1 cytotoxicity. Those cells also were positive by immunofluorescence in a speckled pattern over cell bodies and processes. Cell lines negative for Thy-1 by cytotoxicity absorption were also negative in immunofluorescence assays. The possible function of Thy-1, an antigen shared by T-lymphocytes, developing muscle (Lesley and Lennon, Nature, in press), neurones and glia is open to speculation.

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1263 RESISTANCE TO THE ABSORPTION OF CEREBRO-SPINAL FLUID. James A. Love*, Animal Care Centre, Dalhousie University, Halifax, Nova Scotia and Ronald A. Leslie, Dept. of Anatomy, Dalhousie University, Halifax, Nova Scotia.

The absorption of cerebro-spinal fluid (C.S.F.) has been shown to depend directly on the difference between the subarachnoid space pressure and the sagittal sinus pressure and indirectly on the resistance to absorption which normally resides in the arachnoid villae. It is generally assumed that the resistance does not change over a wide range of intracranial pressures although most authors recognize that at higher intracranial pressures the relationship between pressure flow and resistance becomes less precise.

We have investigated this phenomenon in cats and have found that the resistance to absorption decreases when the intracranial pressure exceeds 20-22 mm Hg. Furthermore, this reduction is maintained to normal intracranial pressure. The reduction in resistance is brought about by the induction of alternate absorption mechanisms which have different opening and closing pressures. The capacity and resistance to absorption of the alternate absorption system have been measured and are similar to those of the arachnoid villae. The potential of alternate absorption mechanisms for C.S.F. is great but the demonstration of such mechanisms has been open to question. We have provided physiological evidence for the presence of these mechanisms and have measured some of their characteristics.

1264 CEREBROSPINAL FLUID (CSF) L LYMPHOCYTES IN MULTIPLE SCLEROSIS (MS) PATIENTS AND NORMAL HUMANS. Roger M. Morrell, Neurology Service, VAH, and Dept. Neurology, Baylor College of Medicine, Houston, TX 77030.

Human L lymphocytes have been identified in peripheral blood, tonsils and lymph nodes in health and disease. L lymphocytes have not been identified in CSF, either in health or disease. This is the first report of their identification in normal subjects and in patients with MS, either untreated or treated with oral steroids (Medrol^{R}, 32 mg., p.o., alternate day).

Human L lymphocytes have membrane-labile IgG determinants and can be distinguished from T cells, B cells and monocytes. L cells form rosettes with human erythrocytes sensitized with IgG(EA). They do not form rosettes with sheep RBC (E), or E sensitized with IgM and mouse complement (EAC). They do not have membrane-incorporated Ig(SmIg), are not phagocytic, and are not stained by non-specific esterase.

L lymphocytes from 10 normal (non-systemic diseases not affecting the CSF, for example, herniated intervertebral disc) and 20 MS subjects were separated from T lymphocytes (E+, SmIg-, EAC-, EA-) and B lymphocytes (E-, SmIg+, EAC+, EA-) by rosetting techniques. L lymphocytes responded poorly to phytohemagglutinin, pokeweed mitogen, tuberculin and streptokinase/streptodornase. They were poor responders in mixed lymphocyte culture (MLC). L and B, but not T lymphocytes were good stimulators. In studies of antibody-dependent lymphocyte cytotoxicity using human lymphocytes sensitized with alloantibodies as target cells, L lymphocytes were effective killers, while T cells, B cells, and monocytes were not.

L cells were more numerous than B cells in normal CSF (ratio 1.7 : 1); the converse was observed in untreated MS CSF (ratio 1 : 18). Steroid-treated patients had decreased numbers of B cells, but only those with lymphopenia had decreased L lymphocytes.

Distinctive surface characteristics, function, anatomical distribution and numerical variation among normals, untreated, and steroid-treated MS patients provide evidence for a CSF L lymphocyte population. The role of this cell type in normal immunity or neuro-immunopathology is not known.

1265 PATHOPHYSIOLOGIC CORRELATIONS IN ABNORMAL CEREBROVASCULAR PERMEABILITY. K. Nishimoto*, H. Pappius*, M. Wolman*, M. Spatz and I. Klatzo. Lab. Neuropath. & Neuroanat. Sci., NIH, Bethesda, MD 20014.

Air embolism as well as occlusion of common carotid artery in Mongolian gerbils served as experimental models to correlate various parameters of the increased cerebrovascular permeability.

Disturbances of the blood-brain barrier (BBB) were evaluated with Evans Blue, sodium fluorescein, and C^{14} sucrose tracers. Changes in permeability of cerebral vasculature to these tracers were correlated with the regional cerebral blood flow (CBF), assessed radioautographically with C^{14} antipyrine method. The BBB changes were also correlated with regional glucose utilization demonstrated radioautographically with the Kennedy technique (Science, 187, 850, 1975). Water and electrolytes were measured in brain tissue samples taken from normal regions and those showing disturbances in cerebrovascular permeability.

Small amounts of air injected into the internal carotid artery resulted within a few minutes in a breakdown of the BBB, whereas changes in cerebrovascular permeability following ischemic occlusion appeared after a delay and according to the principle of the maturation phenomenon described earlier (Acta Neuropath. 32, 209, 1975).

The abnormal permeability to various tracers showed a certain selectivity favoring a longer passage of smaller molecules.

Both types of experiments demonstrated areas of increased and decreased deoxyglucose utilization.

Collected data from various parameters of experimentation will be presented and discussed.

1266 A NEW MODEL FOR DEMYELINATION WITH CONDUCTION DEFICIT IN THE FROG. Terrence L. Pencek, Brenda R. Eisenberg*, Charles L. Schauf, and Floyd A. Davis . Depts. Neurolog. Sci., Pathology, and Physiology, Rush University, Chicago, Ill. 60612.

A segmental lesion has been produced in vivo in the frog, Rana catesbeiana, by opening the perineurium in the sciatic or peroneal nerve. This has been studied by electrophysiological methods and by light and electron microscopy morphometric techniques. A similar perineurial lesion has been reported to induce focal demyelination and remyelination in rats (Brain Research 96:323). In frogs kept at 22-23°C opening the perineurium by careful surgical technique induces edema within 3-5 days, while no visible evidence can be seen in the sham operated control leg or in frogs kept at 13°C. The edema is localized to the perineurial lesion. Conduction velocity measured in vivo is decreased an average of 62% (n=7) across the lesion and not affected 1 cm. distal to the lesion. The nerve bundle cross sectional area in the experimental nerve bundle was 0.99± .15mm^2 and in the control was 0.39± .15mm^2 (n=6). Experimental nerves also had an increase in blood vessel number and size. In the lesion area, the density of fibers was decreased because of the edema. The myelin of the large nerve fibers shows extensive whorling and splitting. The nature of the lesion and conduction velocity deficit will be discussed.

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1267 PRODUCTION OF LARGE VOLUMES OF MOUSE ANTI-MOUSE BRAIN ANTIBODIES IN INDIVIDUAL MICE. Charles See* (SPON: D. C. Wood). Psychobiology Program, Dept. Psychology, Univ. Pittsburgh, Pittsburgh, PA 15260

A major disadvantage in utilizing mice to generate antibodies is the small yield of antiserum obtained from an individual mouse. Munoz (Proc. Soc. Exp. Biol. Med. 107: 163, 1957) originally reported that the yield of antibodies from mice could be substantially increased by inducing ascitic fluid containing antibodies. However, the percentage of mice responding was relatively small. Recently, Tung, et al., (J. Immunol. 116: 676, 1976) reported an improved method for eliciting large volumes of ascitic fluid containing substantial amounts of anti-protein antibodies in about 90% of the mice innoculated. A high volume ratio of adjuvant to antigen solution (9:1) and the volume used per innoculation (0.2ml) were shown to be critical factors in producing large amounts of high titer ascitic fluid.

The present study used this improved immunization procedure to generate precipitating mouse anti-mouse brain antibodies. Swiss Webster mice were innoculated on day 0, 14, 21 and 28 with a 0.2ml of an emulsion of complete Freund's adjuvant and antigen (0.2mg brain homogenate in saline) in a volume ratio of 9:1. The ascitic fluid was tapped repeatedly beginning seven days after the last injection. This immunization protocol elicited significant quantities of ascitic fluid in greater than 80% of a large number of mice injected. The volume varied with the age of the mice with the older mice producing larger volumes. The average mortality rate before significant amounts of ascitic fluid were produced was about 15%. Two precipitin lines occurred when a 1% Triton X-100 brain extract was reacted with the ascitic fluid by double immunodiffusion. All batches of ascitic fluid produced were positive for these two precipitin lines. No precipitin lines occurred when the ascitic fluid was reacted with aqueous brain extracts. One of the precipitin lines was brain specific. This precipitin line did not occur when the ascitic fluid was reacted with 1% Triton X-100 tissue extracts of equal protein concentration from 10 other mouse tissues. Also, no reaction was observed with mouse serum.

This immunization protocol offers several advantages over more conventional procedures for generating large amounts of anti-brain antibodies.

1268 IMMUNOLOGICAL CHARACTERIZATION OF CENTRAL AND PERIPHERAL NICOTINIC CHOLINERGIC ^{125}Iα-BUNGAROTOXIN (α-BGT) BINDING SITES. R.C. Speth*, J.M.Lindstrom, F.M.Chen* and H.I.Yamamura. Univ. Ariz., Tucson, AZ 85724; Salk Inst. San Diego, CA 92112

Recent observations have suggested that myasthenia gravis is an autoimmune disease involving nicotinic acetylcholine receptors (NAChR) of muscle. The lack of any apparent direct CNS effects in myasthenia gravis could indicate that NAChR is either not important to CNS function, that NAChR in the CNS is immunologically distinct from that in muscle, or that the NAChR antibodies do not cross the blood brain barrier.

Previous studies in this laboratory and others have demonstrated pharmacologically specific, saturable, high affinity binding sites for ^{125}Iα-BGT characterized as NAChR.

To test whether central and peripheral α-BGT binding sites are immunologically similar, two series of experiments were performed. For the first series, purified NAChR from E. electricus was used to immunize rats. Immunized rats had 87.3 ± 10% of their muscle NAChR bound with antibody (Ab) while control rats had background values of 0.12 ± 1.5% of their muscle NAChR bound with Ab. Of the immunized rats, 82% were clinically myasthenic. The ^{125}Iα-BGT binding sites of muscle and brain, and the muscarinic antagonist [^{3}H]quinuclidinyl benzilate (QNB) binding sites of control and E. electricus immunized rats were assayed.

Tissue	Binding site	Control	Immunized
Rat Muscle	^{125}Iα-BGT	41.7±0.99pmole/rat	18.3±1.1pmole/rat
Rat Brain	^{125}Iα-BGT	1.88±0.06pmole/g	1.75±0.03pmole/g
Rat Brain	^{3}H-QNB	59.9±1.4pmole/g	57.2±0.9pmole/g

In a second series, we examined the antigenicity of Triton X-100 solubilized ^{125}Iα-BGT binding sites derived from rat muscle and brain to sera obtained from animals immunized with purified NAChR derived from E. electricus or T. californica.

Serum	Tissue	% of ^{125}Iα-BGT binding sites precipitated
Rat anti E. electricus	rat muscle	50
	rat brain	0
Rat anti T. californica	rat muscle	104
	rat brain	0.4-2.1
Rabbit anti T. californica	rat muscle	16
	rat brain	2

These results suggest that there is little or no cross reactivity between ^{125}Iα-BGT binding sites derived from rat muscle and brain. This indicates immunological differences in toxin binding components derived from these two areas. Supported by grants from MDA, NIH #NS-11323 NIMH # MH-25257 and RSDA to H.I.Yamamura.

1269 HISTOPATHOLOGIC EFFECTS OF PENETRATING IMPLANTS ON THE CEREBRAL CORTEX. Suzanne S. Stensaas and L. J. Stensaas. Depts. of Anat. and Physiol., Col. Med., Univ. of Utah, Salt Lake City, UT 84132.

Histopathological changes of the cerebral cortex in response to small, penetrating metal and non-metal implants were analyzed with light and electron microscopy. The needle-shaped implants were left in place during all stages of histological preparation and embedded in plastic together with the cortex. Semi- and ultrathin sections through the brain-implant boundary were classified as non-reactive, reactive, or toxic, according to the reactive cellular constituents. Among the non-reactive materials were many plastics and metals such as aluminum, gold, platinum, and tungsten. The boundary of these implants displayed little or no gliosis and normal neuropile with synapses within 5 μm of the implant's surface. The boundary of reactive materials such as tantalum or silicon dioxide was marked by multinucleate giant cells and a thin capsule (10 μm) of connective tissue. Toxic materials such as iron and copper were separated from the cortical neuropile by a capsule of cellular connective tissue and a zone of astrocytosis. Cobalt, a highly toxic material, produced more extensive changes in the zones of connective tissue and astrocytes. Implant times ranged from 50 to 723 days. These results indicate that a variety of materials are well tolerated by the brain and could be used in the fabrication of neuroprosthetic devices.

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1270 EFFECTS OF CRUSH INJURY AND MYELIN INDUCTION IN SPINAL NERVE ROOTS OF DYSTROPHIC MICE. Charles A. Stirling*, (SPON: Theodore A. Slotkin). Dept. Physiol., Med. Sch., Univ. Bristol, Bristol, England.

Dystrophic mice, 129/ReJ dy/dy, have an inherited abnormality of their peripheral nervous system apparent as a lack of myelin or any other Schwann cell wrapping, termed amyelination, in localized regions of individual nerves (Stirling, J.Anat. 119: 169, 1975). The genetic mechanism which produces this lesion is not known, but two, of a number, of potential hypotheses are: 1. that the Schwann cells are irreversibly turned off from further division at too early a developmental time and 2. that the recognition of axon membrane by Schwann cell membrane is impeded in the amyelinated regions. These two hypotheses have been tested and evidence is presented against both (Stirling, Brain Res. 87: 130, 1975).

Dorsal roots of anaesthetized dystrophic mice about one month old, 6 to 15 g, were partially crushed with a pair of fine pointed forceps. The animals were then allowed to recover for between 1½ and 45 days. Three animals were labelled with tritiated thymidine for autoradiography. All the animals were perfusion fixed for electron microscopy, autoradiographs were made for light microscopy on plastic thick sections with adjacent sections used for E.M.

The Schwann cells in crush traumatized roots were found to be capable of cell division for up to at least eight days recovery time and at least 3 mm distance both proximal and distal to the site of injury. Control side, non-crushed, roots showed no labelled cells and no other signs of cell division.

In the crush regions and for varying distances either side new Schwann cell wrapping of axons was evidenced both by many more axons being surrounded and by newly dividing Schwann cells being involved. In the longer recovery animals, greater than ca. 7 days, axons were frequently found with multiple layers of Schwann cell wrapping, very early myelin condensation or a few myelin lamella. The amount of new myelin formed appeared to increase with recovery time up to 45 days, the longest time examined, but this could not be quantitated due to inevitable small differences in crushing and surgical procedure. The control side roots remained normal amyelinated dystrophic roots with little or no noticeable morphological effect from the experimental procedures.

Dystrophic Schwann cells can therefore divide given an adequate stimulus and they can recognize dystrophic axons as objects to myelinate.

Supported by M.R.C.

1271 FASCICULAR DISTRIBUTION OF SPINAL CORD EDEMA PRODUCED BY EXPERIMENTAL CORD CONTUSION. Franklin C. Wagner,* William B. Stewart* and William F. Collins. Dept. of Neurosurgery and Physiology, Yale Univ. Sch. of Med., New Haven, Conn. 06510.

It has been reported that experimental contusion of the spinal cord produces an extravasation of proteins from vessels in the gray matter at the site of the lesion (Green, B.A. and F.C. Wagner, Surgical Neurol. 1:98-101, 1962). At subsequent intervals after trauma there is increasing involvement of the surrounding white matter. With cold lesions of the cerebral cortex, it has been demonstrated that edema spread is primarily through the white matter to adjacent gyri (Klatzo, I.; J. Miguel and J. Ostenasek, Acta Neuropath. 2:144-160, 1962). With Evans Blue as a marker, Griffiths and Miller (J. Neurol. Sci. 22:291-294, 1974) have shown centrifugal and longitudinal spread of gray matter edema in the dorsal and lateral white columns following cord contusion. The purpose of the present experiments was to determine to what extent longitudinal spread of edema occurred in the white matter of the spinal cord, since the primarily longitudinal arrangement of the long tracts might afford a more simplified analysis of the mechanisms involved.

Thirty-three cats were injected with fluorescein-labelled albumin (FLA) or fluorescein-labelled dextrans (FLD) with molecular weights of 20,000 to 150,000. The thoracic cord was subjected to a contusion of 20 gms from 25 cm. The extent of the edema was examined in animals sacrificed 8 hours after trauma. Proximal and distal to the lesion the edema had a fascicular pattern. There was edema primarily in the lateral and ventromedial white columns. These areas were not immediately adjacent to the grey matter, suggesting that the edema did not spread from the gray at that cord level. The distance of spread in the longitudinal direction was similar for the FLA and FLD molecules. The maximum area of fluorescence typically occurred several millimeters outside the hemmoraghic portion of the lesion.

The distribution of fluorescence in these experiments suggests that several mechanisms may be involved in the development and spread of edema. Two of these may be bulk flow and alteration of blood flow. Experiments are currently being performed to assess the possible role of these mechanisms in spinal cord trauma.

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1272 INHIBITION OF REMYELINATION BY CYTOSINE ARABINOSIDE FOLLOWING VIRAL-INDUCED DEMYELINATION. Leslie P. Weiner, Stephen A. Stohlman.* Dept. Neur., Sch. Med., USC, Los Angeles, CA 90033.

Infection of mice with the JHM strain of mouse hepatitis virus causes demyelination as a result of cytolytic infection of oligodendroglia. In recovery, animals show remyelination beginning at day 21 post infection (PI). Recently, Herndon et al (Science 195: 693-694, 1977) have demonstrated by utilizing electron microscopic autoradiographic studies with ^{3}H-labeled thymidine that cells associated with remyelination are newly generated oligodendroglia.

This current study shows that cytosine arabinoside (ara-C), a potent inhibitor of cell multiplication and DNA synthesis, inhibits remyelination when injected into animals twice daily from day 15 to 20 PI. 4 week old outbred Swiss mice were inoculated intracerebrally with 10 LD_{50} doses. 40 percent of animals died within 10 to 12 days, and all survivors, showing no clinical signs, had evidence of active demyelination. By day 15 PI, no evidence of infectious virus could be found, and animals received 50 mg/kg of ara-C twice daily by intraperitoneal (IP) injection. At the same time, mice received 6 µc/g of ^{3}H-labeled thymidine (S.A. 40.2 c/m mole) every 12 hours. Animals receiving virus alone showed high levels of incorporated ^{3}H-thymidine measured as CPM/g of tissue and showed by autoradiography labeled CNS and inflammatory cells. The animals receiving virus and ara-C had 75% less incorporation of ^{3}H-thymidine than animals with virus alone but still more than controls inoculated with suspensions of normal mouse brain. At 20 and 34 days PI, virus-inoculated animals had active remyelination as well as a marked inflammatory response. Animals receiving virus and ara-C had little if any inflammatory cells and denuded axons as late as 14 days post ara-C (34 days PI). This absence of remyelination was accompanied by a sparsity of oligodendroglia.

From this data, it would appear that oligodendroglia proliferation is important in the myelin repair following virus-induced demyelination as evidenced by the interference of remyelination with the use of DNA inhibitors. Other mechanisms by which ara-C might interfere with remyelination will be discussed.

Supported by The Kroc Foundation.

1273 INTRAHYPOTHALAMIC INJECTION OF DIFFERENT IMMUNOLOGICAL REACTION SYSTEMS INDUCE SPECIFIC CHANGES IN APPETITIVE BEHAVIORS. C.A. Williams and N. Schupf*SUNY, Purchase, N.Y. 10577, NYU Medical Center, New York, N.Y. 10016

ARB, a rabbit antibody produced against a membrane fraction of rat brain, was shown to depress drinking in deprived animals when introduced into the perifornical hypothalamus. This effect was identical to that produced by a complete exogenous reaction system, e.g. antiovalbumin and ovalbumin (Science, 196,328,1977). Since no common immunological event could be postulated for these similar results, the modes of action of these systems were tested utilizing a more sensitive assay based on the pharmacological induction of appetitive behavior in sated animals. Rats were prepared with cannulae in the perifornical hypothalamus, a region where direct chemical stimulation with norepinephrine (NE) is known to elicit excessive eating, to counteract carbachol induced drinking,and to depress natural thirst. Direct chemical stimulation with carbachol at the same site will elicit excessive drinking, block NE induced eating, and decrease eating in response to food deprivation.

Pretreatment with the exogenous antigen system elicited food intake in saline control animals, increased the eating response to NE treatment and suppressed the drinking response to carbachol treatment (Neurosci. Abstr., #1061,1975). In contrast, aRB did not modify the eating effect of NE or the drinking effect of carbachol. Nor did it induce appetitive behaviors in control animals not stimulated by drugs. However, pretreatment with aRB resulted in a significant increase of food intake in carbachol stimulated animals and a significant increase of water intake in NE stimulated animals. These effects demonstrate that aRB decreases the mutually antagonistic actions of NE and carbachol at this site.

The results of these experiments indicate that aRB and the defined reaction systems do not have similar modes of action on on the neural substrates mediating appetitive behavior. Reactions of extracellular soluble antigens with antibody in other tissues is known to cause noncytotoxic release of biogenic amines. In the brain model, the behavioral effects are consistent with the release of NE, although another mechanism resulting in the activation of NE dependent pathways would suffice. Antibody against membrane antigens of the brain, however, would be expected to react directly with cell surface sites. We propose that aRB acts to selectively impair the function of interneurons mediating adrenergic-cholinergic antagonism.

1274 PENETRATION OF A TRACER THROUGH CELLULAR ELEMENTS AROUND A CANNULA IMPLANTED IN BRAIN. Lawrence R. Williams* and Terence H. Williams. Dept. Anat., Coll. Med., Univ. of Iowa, Iowa City, IA 52242.

In the course of experiments requiring an implanted cannula for repeated, localized injection of agents into the septal region of the rat brain, information was sought about the tissue reaction around the cannula using electronmicroscopy. Horseradish peroxidase (HRP), Sigma Type VI (M.W. 40,000), was used as a tracer to determine whether or not the cells around the cannula would present a barrier to larger molecules. A microknife wound, made through the cannula, provided the opportunity to study the cellular changes after extraction of the knife.

Shultz and Willey (J. Neurocytol. 5:621, 1976) described the sheath of monocyte-derived, foreign body giant cells that surround electrodes 30 days after implantation. These investigators did not report on earlier time periods and a marker was not employed.

A stainless steel, 23 gauge cannula was used. Microknife wounds, 1 mm in depth, and injections of 1 µl saline or 0.2 - 1.0 mg/µl HRP at a rate of 0.05 µl/30 sec. were made via the cannula. The HRP or saline was injected 30 min. before sacrifice by perfusion fixation. Between the cannula implantation and sacrifice was an interval of 1, 3, 5, 7, 14 or 21 days.

Edema, neuron damage and phagocytosis characterized the tissue surrounding the cannula at 1-3 days survival. At 3-5 days, reactive astrocytes, monocytes and leptomeningeal cells appeared between the cannula and adjacent tissue. These cells lined most of the lesion by 7 days. At 14-21 days, the cells around the cannula were mainly monocytes and giant cells. Both kinds of cells formed a mutlilayered lining with complexly folded surface membranes. The tissue reaction around the microknife wound was similar to the reaction around the cannula.

In tissue sections, HRP reaction product appeared as a 1-3 mm wide band around the cannula. Individual variation in HRP diffusion patterns was observed, but there was a trend towards a decreased reaction product band width with increased survival time. HRP reaction product was localized primarily in the extra-cellular space (ECS) between these lining cells and in the ECS of the adjacent neuropil.

This evidence indicates that the cells surrounding the cannula do not present a substantial barrier to injected HRP during the time periods studied. The trend toward reduced penetration of the marker with increasing time after cannulation may be related to the increasing thickness and complexity of the cellular lining observed around the cannula. (Supported in part by Anatomy Training Grant GM00148 and NS11605 to T.H.W.)

NEUROTRANSMITTERS

1275 ELECTRICAL STIMULATION CAUSES INCREASED ACETYLCHOLINE SYNTHESIS IN A CRUSTACEAN SENSORY NERVE. Anthony Auerbach* (SPON: J. Sidie). Biology Dept., University of Oregon, Eugene, OR 97403.

The paired posterior stomach nerves (PSN) innervate the gastric mill region of the decapod crustacean stomach. In the crab Cancer magister it contains about 60 bipolar, anatomically homogeneous neurons with discrete dendrite, cell body, and axon regions. The PSN synthesizes acetylcholine (ACh) from choline but not any of the known transmitters derived from tyrosine, glutamate or tryptophan. PSN neurons also contain high choline acetyltransferase (CAT) activity. Thus, PSN neurons, like many other crustacean sensory systems, use ACh as their transmitter. Dendrite, soma, and axon regions all take up ^{3}H-choline and produce three major metabolites: phosphorylcholine (PCh), betaine, and ACh.

Kinetic analysis of choline transport shows both a low affinity (K_m=94 μM) and a high affinity (K_m=2 μM) component. The high affinity system predominates only when [choline] is less than 2 μM. The ratio, ACh synthesis/choline uptake, increases with decreasing exogenous [choline] in a manner consistent with coupling of ACh synthesis to high affinity transport. No such coupling is apparent for betaine or PCh. A PSN electrically stimulated (10-15 Hz) for 2-8 hrs and subsequently incubated in ^{3}H-choline and 10^{-5}M eserine synthesizes more ^{3}H-ACh than an unstimulated control PSN from the same animal. With 20 μM ^{3}H-choline, the stimulated/unstimulated (S/U) ^{3}H-ACh synthesis ratio was 1.72±0.18 (mean±SEM, n=6), while paired unstimulated controls showed the ratio 0.97±0.08 (n=4). With 1-2 μM ^{3}H-choline, the S/U ratio for ACh synthesis was 2.68± 0.27 (n=3) and a significant increase in total choline uptake was also observed (S/U=1.68±0.12; n=3). The increased ACh synthesis may result, in part, from activation of high affinity choline transport upon stimulation. However, stimulated PSNs also show increased CAT activity in cell-free assays, suggesting that electrical activity may alter other aspects of choline metabolism. These effects probably depend directly on action potentials since 1) the PSN is a primary sensory system devoid of synaptic input, and 2) antidromic stimulation produces the same metabolic changes. The metabolism of this electrical-metabolic coupling is under investigation. (Supported by NIH grants GM 00336 and NS-10614 (to D. Barker), and Sigma Xi.)

1276 SUBSTANCE P TERMINALS FORM SYNAPTIC JUNCTIONS IN SPINAL CORD. R. P. Barber*, J. E. Vaughn, J. R. Slemmon*, E. Roberts, and S. E. Leeman. Division of Neurosciences, City of Hope National Medical Center, Duarte, California 91010; and Department of Physiology (LHRRB), Harvard Medical School, Boston, Massachusetts 02115.

Molecules which react with antiserum to substance P have been localized in light and electron microscopic preparations of rat spinal cord using an immunoperoxidase method. Substance P positive (SP+) product is highly concentrated in dorsal horn laminae I-II, Lissauer's tract, a small nucleus in the dorsal portion of the lateral funiculus, and in a discrete patch just ventral to the central canal. Moderate accumulations of SP+ product are observed in portions of laminae III-V, lamina X, and in a narrow zone bordering fasciculus gracilis that expands ventrally to include nucleus cornucommisuralis dorsalis and nucleus dorsalis. The rest of the spinal grey matter exhibits extremely sparse staining. Light microscopic observations suggest that SP+ product is concentrated in fine axons and collaterals which display densely-stained varicosities and terminal puncta. These structures are closely associated with neurons and blood vessels. Electron microscopic observations reveal that certain axons and terminals contain accumulations of SP+ product associated with large, dense-core vesicles. Lesser amounts of SP+ product are sometimes associated with small, clear synaptic vesicles surrounding the dense-core vesicles. Such terminals commonly form axodendritic and axosomatic synapses, but the depositions of SP+ product are often not concentrated precisely at the "active sites" of these synaptic junctions. SP+ axon terminals also make contacts with astrocytic processes, including those which form endfeet around blood vessels. A primary afferent origin of some of the SP+ fibers and puncta is supported by the observation that ipsilateral, dorsal rhizotomies markedly reduce SP+ staining in laminae I-II. However, this staining is not entirely abolished by the lesions, and there is no obvious loss of SP+ staining in other parts of the spinal grey matter. Our findings suggest that substance P could participate in neural functions which are both spatially limited to synaptic junctions and more diffusely distributed via an involvement of the glio-vascular and ventricular system. (Supported by USPHS grants NS 12116, NS 09578, and AM 16510).

1277 POSTSYNAPTIC CORRELATES OF BARBITURATE EFFECTS OBSERVED IN TISSUE-CULTURED MAMMALIAN NEURONS. J.L. Barker and R.L. Macdonald, LNP, NINCDS and BBB, NICHD, Bethesda, Md. 20014.

Dissociated neurons derived from mouse fetal spinal cord and grown in tissue culture have been used to investigate the effects of barbiturates on neuronal membrane properties and amino acid pharmacology. Perfusion coupled with intracellular recordings revealed that anesthetic concentrations of pentobarbital (PB) reversibly abolished all synaptic activity, but did not render individual cells inexcitable, while anticonvulsant concentrations of phenobarbital (PhB) did not alter spontaneous activity, but markedly attenuated picrotoxin-produced convulsant activity.

The post-synaptic membrane mechanisms underlying barbiturate pharmacology were studied by adding Mg^{++} to the plate to suppress synaptic activity and by iontophoretically applying barbiturates and amino acids. PB directly and reversibly increased membrane conductance in a dose-dependent manner, the limiting slope of the log-log dose-response curve being about 2. These effects were Cl-dependent and antagonized by picrotoxin and penicillin. PhB either did not directly affect membrane properties (n= 16 cells) or produced small effects at large currents (>200 nA). PB and PhB potentiated GABA responses but not those of glycine or β-alanine, one molecule of either barbiturate being involved in the modulation. PB was 3-5 times more potent, prolonged GABA kinetics and lowered the limiting slope of the log-log dose-response curve from about 2 to about 1.5. PhB enhanced GABA with little change in kinetics. The results suggest that PB is GABA-mimetic and in some way participates in the GABA response, thus lowering GABA cooperativity. Although analysis of double-reciprocal plots was difficult due to the apparent change in GABA cooperativity, similar y-intercepts of the plots suggest no increase in the "maximum effect" of the GABA. PB and PhB reversibly depressed glutamate (GLU) depolarizations without altering their kinetics, with one molecule of either barbiturate being required. PB was about 3-5 times more potent than PhB. PB but not PhB indirectly depressed GLU by shunting the membrane. Analysis of the double-reciprocal plots indicated that PB and PhB antagonized GLU through a non-competitive mechanism. The threshold for enhancement of GABA and depression of GLU by the barbiturates occurred at about the same iontophoretic current. Similar differential effects on membrane properties and amino acid responses were made with the anesthetics, secobarbital and DMBB, and the anti-convulsant mephobarbital. The results suggest that barbiturate anesthetics anesthetize by directly activating GABA receptors, by potently enhancing and prolonging GABA-mediated inhibition and by depressing glutamate excitation. Anti-convulsant effects appear to result from less potent, though similar modulation of GABA and glutamate effects without direct activation of GABA receptors.

1278 EFFECT OF THYROTROPIN RELEASING HORMONE ON RAT HYPOTHALAMIC AND SEPTAL NEURONS: COMPARISON WITH NOREPINEPHRINE AND ACETYLCHOLINE. A.L. Beckman and A. Winokur.* Depts. of Physiology and Psychiatry, School of Medicine, Univ. of Pa., Philadelphia, Pa. 19104

Recent evidence has demonstrated that hypothalamic releasing factors exert an influence on the firing rate of neurons within diverse areas of the brain, suggesting a possible role as neurotransmitters. In the present study, we compared the effect of thyrotropin releasing hormone (TRH) with that of norepinephrine (NE) and acetylcholine (ACh), two putative neurotransmitters, on the firing rate of neurons in the cortex, preoptic/anterior area of the hypothalamus (PO/AH), and the septal area of urethane-anesthetized, male Sprague Dawley rats. Five-barreled micropipettes were used to record extracellular single unit discharges and to iontophoretically apply TRH (0.01M), NE (0.25M), and ACh (0.25M). Only those cells that responded to at least one of the test compounds (TRH, NE or ACh) and that failed to respond to current flow (delivered through a 0.5M NaCl-filled barrel) were included in these results. Twelve cells in the cerebral cortex were tested and none responded to TRH. However, in the PO/AH and septal area, TRH influenced the firing rate of 29 out of 43 cells (67%). The effect was uniformly one of inhibition. The majority (90%) of the cells inhibited by TRH were also inhibited by NE (N=26; 3 cells were unaffected by NE) but, by contrast, showed variable responses to ACh. These data show that TRH exhibits similar effects to NE on almost all TRH-sensitive neurons tested. The mechanism underlying these parallel effects has yet to be defined. (Supported by USPHS grant NS10597 and NIH RCDA award MH00044.)

1279 TRANSPORT AND SECRETION OF NEURAL PEPTIDES WITHIN THE CNS OF APLYSIA. R. W. Berry. Dept. Anat., Northwestern Med. Sch., Chicago, IL 60611.

Many molluscan neurons produce large quantities of low molecular weight proteins, and the somatic and axonal metabolism of these compounds have been studied intensively. However, the function of these abundant peptides is not known. Indirect evidence suggests that they are neurosecretory, but only in the case of the egg-laying hormone secreted by the bag cells of Aplysia has release been directly demonstrated (Arch, J. gen. Physiol., 59, 47, 1972). To test the assumption that such peptides are actually destined for secretion, we isolated the intact central nervous system of Aplysia in such a way that the circumesophageal ganglia could be exposed to tritiated leucine while the pleuro-visceral connective nerves and the abdominal ganglion were bathed in a large excess of unlabeled leucine. Under these conditions, labeled proteins are transported into the connective nerves and reach the abdominal ganglion within 24 hr. The predominant group of transported proteins consists of peptides smaller than 18,000 daltons. Electrical stimulation of the connective nerves releases labeled transported protein from the abdominal ganglion, and this release is inhibited in low Ca^{++}:high Mg^{++} media. The labeled protein that remains in the ganglion after stimulation is deficient in low molecular weight species. Thus, peptides synthesized by neurons of the circumesophageal ganglia are transported to the abdominal ganglion and undergo release there. In view of a previous demonstration that pharmacologically active peptides can be extracted from the circumesophageal ganglia (Ifshin, et al., Nature, 254, 72, 1975), the present data suggest that such neuroactive peptides could reach their targets not as circulating hormones, but via a synaptic mechanism.

Supported by NS 11519 from NINCDS.

1280 EXCITATORY EFFECTS OF THRYROTROPIN RELEASING HORMONE IN CAT SPINAL CORD. Gene Boyer* and Barrett R. Cooper* (SPON: James L. Howard). Dept. of Pharmacol., Wellcome Res. Labs, Research Tri. Pk., N.C.

Thyrotropin releasing hormone (TRH) is a tripeptide localilized in hypothalamus and also reported to have a widespread distribution through the CNS[1]. We found TRH (10 mg/kg i.v.) to produce a generalized increase in muscle tonus as evidenced by highly activated EMG recorded from flexor and extensor groups throughout the skeletal musculature. EMG activation occurred within 10 sec after TRH injection via indwelling venous cannula in restrained but otherwise untreated cats. TRH similarly increased the amplitude and frequency of the EMG in cats after midcollicular decerebration or high spinal cord transection. Studies in C_1 spinal cats revealed that TRH could transiently (3-5 min) increase EMG activity at a dose as low as 0.5 mg/kg i.v. Doses ranging from 5-10 mg/kg i.v. however, elicited a stimulation of EMG activity lasting at least 2 hrs and beyond. Curiously, tolerance rapidly developed to repeated injections of TRH. For example, 2 mg/kg i.v. increased EMG activity up to 100 fold and had measurable effects for 10 min; whereas a second dose of 2 mg/kg i.v., given 30 min after the first injection, produced only a 2 fold increase and had effects for 10 min; and a third dose of 2 mg/kg i.v. 30 min later elicited only a barely measurable response. Direct recording from L_6 or L_7 spinal cord ventral roots after laminectomy, bilateral rhizotomy of both L_6 or L_7 dorsal and ventral roots, and cord transection at C_1 revealed that spontaneous nerve action potentials occurred at a high rate after i.v. injection of TRH. Computer averaging of ventral root potentials evoked by electrical stimulation of dorsal roots in these cats showed that TRH increased the amplitude of the monosynaptic potentials and both increased the amplitude and shortened the latency of the polysynaptic potentials. None of these effects of TRH was produced by injection of T_3 or the constituent amino acids of TRH. These results and the recent discovery of TRH in the anterior horn[2] suggest that TRH may function in spinal cord neural transmission.

1. Winokur, A. and Utiger, R. D. *Science*, 185 (1974) 265-267.

2. Kardon, F. C., Winokur, A. and Utiger, R. D. *Brain Research*, 122 (1977) 578-581.

1281 ELECTROPHORETIC AND IMMUNOCHEMICAL CHARACTERIZATION OF CHOLINE ACETYLTRANSFERASE FROM TORPEDO. C. Brandon* and Jang-Yen Wu* (Spon: K. Matthews) Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030

We have previously reported the purification of choline acetyltransferase (CAT), the enzyme catalyzing the biosynthesis of acetylcholine, from the electric organ of Torpedo californica, and shown it to migrate as a single band on SDS-polyacrylamide gels (Trans. Amer. Neurochem. Soc. Vol. 8, page 169, 1977). On non-denaturing 5% gels at alkaline pH, the purified protein migrates as a single band of R_f 0.32. Enzymatic (CAT) activity can be recovered by slicing the gel, and corresponds exactly to the position of the protein as visualized by coomassie staining. Cation-exchange chromatography has been reported by several authors to separate CAT into two charge isozymes (see, e.g., PNAS 70, 3749 (1973); J. Neurochem. 24, 963 (1975). These two isozymes can be separated by electrophoresis of the purified protein on 5% gels at pH 4.2 under non-denaturing conditions, and appear in approximately equal amounts. These isozymes have identical molecular weights on SDS-gels.

Using rabbits, we have obtained an antibody specific to CAT following five biweekly subscapular injections of 10 µg of the purified enzyme in complete Freund's adjuvant. The antisera thus obtained show a single precipitin band on Ouchterlony double diffusion tests against both crude CAT (1% pure) and the purified protein. Using these antibodies, studies on the species and tissue specificity and immunohistochemical localization of CAT are in progress. (Supported in part by Grant NS-13224-02 from NIH).

1282 EFFECTS OF ESERINE AND ESEROLINE ON ACETYLCHOLINE (ACh) UPTAKE IN RAT BRAIN SLICES. Virginia G. Carson, Donald J. Jenden, Ruth A. Booth* and Margareth Roch*. Dept. Biol., Chapman College Orange, CA 92666 and Dept. Pharmacol. & Brain Research Inst., UCLA Sch. Med., Los Angeles, CA 90024.

Eseroline, the phenolic hydrolysis product of eserine, has been reported to be essentially inert as a ChE inhibitor(Hemsworth B.A. and West, G.B., J. Pharm. Pharmacol. 20:406, 1968). Eserine is also a potent inhibitor of ACh uptake in brain slices(Schuberth J. and Sundwall,A., J. Neurochem. 14:807, 1967) and it was of interest to examine whether eseroline retains this property. Eseroline was prepared by basic hydrolysis of eserine in a nitrogen atmosphere followed by extraction into ether and crystallization as the hydrochloride salt (M.P. 214-215°C). Slices of rat brain cortex were incubated at 38°C in Krebs solution containing 1mM ascorbate, 50µM paraoxon and eserine or eseroline at concentration of 10^{-4}, 3 X 10^{-5}, 10^{-5}, 3 X10^{-6}, 10^{-6} or 3X10^{-7} M. Controls were identical except eserine and eseroline were omitted. After preincubating for 15 min, $^2H_3$-acetyl-$^2H_2$-choline (20µM) was added and the tubes were incubated for an additional 30 min. Both slices and supernatent were then analyzed by GCMS for Ch and ACh labelled in either Ac or Ch moiety, in both and in neither. No evidence was obtained of significant hydrolysis and resynthesis of ACh, which would yield a singly labelled product. The uptake ratio (slice/supernatent) of $^2H_3$-Ac-$^2H_2$-Ch in control slices was 2.26 ± .08 (66). Eserine and eseroline were equiactive in inhibiting the uptake, with I_{50} of 1.0 X 10^{-5}M and 1.6 X 10^{-5}M respectively. Neither compound affected the levels of unlabelled (i.e. endogenous) Ch (147 ± 4 (189) nmol g^{-1}) or ACh (31.3 ± 0.7 (191) nmol g^{-1}) or the amounts of endogenous Ch or ACh released into the bath (18.4 ± .4 (191) and no detectable nmol g^{-1} min^{-1} respectively).

We conclude that inhibition of ACh uptake by eserine depends on the eseroline nucleus rather than on the carbamate ester group which is necessary for ChE inhibition. Eseroline may be useful in assessing whether ACh uptake plays a functional role in cholinergic systems. (Supported by USPHS grant MH 17691).

1283 SUPPRESSION OF JAW-OPENING REFLEX BY MORPHINE: THE INVOLVEMENT OF CHOLINERGIC AND DOPAMINERGIC NEUROTRANSMISSION. Samuel H.H. Chan and M.K. Yip*. Dept. Life Sci., Indiana State Univ., Terre Haute, IN 47809 and Dept. Physiol., Univ. Hong Kong, Hong Kong.

The jaw-opening reflex (JOR), elicited by activating the tooth pulp afferents and resulted in the contraction of the digastric muscles, has been used as a model in the study of pain. Chan and Fung (Exp. Neurol. 53:363, 1976) have reported the suppression of this reflex by morphine in rabbits, and have suggested that the opiate may promote the release of neurotransmitters centrally which inhibit the transmission of nociceptive information from the tooth. The present study was undertaken to investigate this possible modus operandi of morphine-elicited analgesia.

Adult rabbits lightly anesthetized with pentobarbital sodium (30 mg/kg, i.v.) were used. Intrapulpal stimulation was delivered via a pair of silver electrodes implanted into the dental pulp of the left upper incisor. The evoked JORs were recorded as EMG signals from the left digastric muscle. Changes in the averaged amplitude of 30 EMGs obtained from an on-line averager, as elicited by constant intensity (3T) dental stimulations, were used as the index of pain response. Drugs were administered directly into the cerebral circulation via a cannulated carotid artery. Animals were maintained on artificial respiration throughout the recording session to eliminate morphine-induced hypoxia.

As previously observed, intracarotid injection of morphine sulfate, at a dose of 3 mg/kg, caused no or only transient inhibition on the JOR. The analgesic effects of morphine, at this dose, were significantly potentiated when physostigmine (0.1 mg/kg), an acetylcholine esterase inhibitor, was introduced 30 min after morphine administration. Such action, indicative of the involvement of cholinergic transmission, can be observed as soon as 3 min after physostigmine was delivered and can endure for at least 20-30 min. Atropine sulfate, an acetylcholine antagonist, given at doses of 1 and 5 mg/kg, nonetheless, was unable to reverse the ongoing inhibitory effects on the JOR.

Morphine, at an analgesic dose (6 mg/kg) as previously demonstrated, was observed to produce no appreciable effects on the JOR in rabbits pretreated, 45-60 min before the recording session, with a dopamine receptor blocker, pimozide (0.5 mg/kg, i.p.). Significant inhibition of the reflex, however, was achieved when physostigmine (0.1 mg/kg) was administered 30 min after morphine injection.

It would seem reasonable to postulate that morphine suppression of JORs may be exerted by promoting a change in cholinergic and dopaminergic neurotransmission. Exactly where morphine may induce such a release of neurotransmitters in the central nervous system is currently being investigated in our laboratories.

1284 A MECHANISM OF ACTION OF BENZODIAZEPINES: EFFECT OF CHLORDIAZEPOXIDE UPON GABA CHEMOSENSITIVITY AND SYNAPTIC ACTIVITY IN SPINAL TISSUE CULTURE. Dennis W. Choi*, David H. Farb*, and Gerald D. Fischbach. Dept. of Pharm., Harvard Medical School, Boston, MA 02115

The mechanism of action of benzodiazepines is unknown. Some indirect evidence suggests an interaction with inhibitory GABA or glycine pathways, but the complexity of the brain has prevented precise analysis. We have determined the effect of chlordiazepoxide (CDPX) in spinal cord cell cultures, where individual neurons can be tested directly.

Dissociated embryonic chick spinal cord neurons survive in sparse cell culture and form extensive synaptic interconnections. Individual neurons were impaled for intracellular recording, and drug responses were evoked by focal microiontophoresis, and/or by pressure ejection from large bore pipettes. All spinal neurons are sensitive to both GABA and glycine. Prior application of bicuculline, or strychnine, reversibly attenuated GABA and glycine responses, respectively.

Prior application of CDPX invariably resulted in a striking potentiation of the GABA response, while having no effect on the glycine response. The potentiation was rapidly reversible, and its magnitude was CDPX dose-dependent (between 10^{-7} M and 10^{-4} M). CDPX alone at these concentrations had no effect on resting potential or input resistance. The GABA dose-response function was markedly shifted to the left by CDPX, but the maximal GABA response was unchanged.

One mM CDPX did not affect high affinity ^{3}H-GABA uptake measured in sister cultures. Induction of GABA release cannot account for the large shift in the GABA dose-response function, because CDPX did not exert a large GABAmimetic effect. The CDPX potentiation of GABA chemosensitivity, therefore, is likely due to a direct postsynaptic action. Since CDPX is not an agonist, it probably does not act directly at the GABA binding site. It may bind to a regulatory site on or near the GABA receptor, altering the receptor's affinity for GABA. Alternatively, the binding of CDPX may alter the coupling between GABA binding and the opening of membrane channels. This type of postsynaptic facilitation may represent a novel drug mechanism.

Stimulus evoked synaptic potentials that are blocked by bicuculline are augmented by CDPX, suggesting that CDPX facilitates the action of endogenously released GABA at GABAnergic synapses. The overall effect of CDPX on ongoing spontaneous synaptic activity is one of marked inhibition. These findings may help explain some of the clinical actions of benzodiazepines on the basis of enhanced GABAnergic inhibition in the CNS.

1285 SOME ACTIONS OF CATECHOL ON DORSAL ROOT RESPONSES IN FROG SPINAL CORD. Robert A. Davidoff, John C. Hackman and David B. Ross*. Neurology Service, V.A. Hospital and Dept. of Neurology, University of Miami School of Medicine, Miami, FL 33152

Catechol is an unusual convulsant agent in that it augments spinal primary afferent depolarization (PAD). This has been attributed to the compound's ability to increase the amount of transmitter released presynaptically by nerve impulses. However, our present experiments--designed to ascertain the nature of catechol's effects on primary afferent fibers--suggest a postsynaptic mechanism of action.

We used the hemisected frog spinal cord continuously superfused with HCO_3^- buffered Ringer's solution maintained at 15°C. Using the sucrose gap technique, synaptic and amino acid-induced responses were recorded from dorsal roots (DR). Catechol, added to the superfusate in concentrations greater than 10^{-5}, consistently and significantly enhanced the amplitude and increased the duration of dorsal root potentials (DRPs) whether generated by stimulation of an adjacent dorsal root (DR-DRP) or of a ventral root (VR-DRP). The latter potential usually increased more in size (300% of control response; 60 min application) than the former (150%). The dorsal root reflex was also facilitated. These effects were only partially reversible after washing.

Although changes in primary afferent membrane potential (usually transient hyperpolarization, 0.5-0.75mV) and excitability (increased amplitude of antidromic spike elicited by Wall's technique) were noted, it is likely that the increased DRPs were caused by a postsynaptic potentialtion of the action of neutral amino acids. These substances--particularly GABA--are thought to be responsible for PAD. Thus, in Ringer's solution containing 10-20mM Mg^{++} (which suppresses synaptic activity) the amplitude of the DR depolarizations produced by GABA, β-alanine, glycine, and taurine were invariably and substantially enhanced (150-350%); durations of these depolarizations were unaffected. Similarly, catechol increased the DR depolarizations evoked by elevated concentrations of K^+ in the superfusate. Furthermore, catechol neither altered high affinity uptake of [^{3}H]GABA by cord slices nor K^+-evoked release of the same compound from similar slices.

These observations suggest that the catechol-induced enhancement of the DRP could be caused by an increased action of synaptically released GABA (or another neutral amino acid) on DR terminals and/or by an increased depolarizing effect of K^+ accumulating in extracellular spaces during the DRP. Therefore, it seems reasonable that a postsynaptic mechanism provides the basis of catechol's ability to enhance PAD. It remains to be determined what relationship this action has to catechol's convulsant properties. (Supported by VA Funds, MRIS 1769).

1286 GABA AS A NEUROTRANSMITTER IN SPINAL CORD CELL CULTURES. David H. Farb*, Dennis W. Choi*, and Gerald D. Fischbach (SPON: N. Kiang). Department of Pharmacology, Harvard Medical School, Boston, MA 02115

A great deal of circumstantial evidence has accumulated that γ-aminobutyric acid (GABA) plays a role in presynaptic and postsynaptic inhibition in the adult vertebrate spinal cord. We are attempting to demonstrate that GABA is a neurotransmitter in embryonic spinal cord cell cultures. Neurons were dissociated from 7 day embryonic chick spinal cords and were either added to established muscle cultures or grown alone. ^{3}H-GABA is accumulated in established spinal cord-muscle cocultures by a high affinity uptake mechanism (Km = 3.7 μM). Autoradiography indicates that about 50% of the neurons (defined by their ability to generate an action potential or receive synaptic input) concentrate the label. Significantly, none of the neurons that innervate muscle are labeled. Newly taken up ^{3}H-GABA was released when the cells were depolarized in 100 mM K^+. Reduction of the Ca^{++}/Mg^{++} ratio reversibly inhibited the stimulated release.

Cultured spinal cord cells synthesize ^{3}H-GABA from ^{3}H-glutamate (products identified by high voltage paper electrophoresis). The Km for glutamate is ca. 150 μM. GABA synthesis did not depend on the presence of non-neuronal cells: formation of ^{3}H-GABA was unchanged when the non-neuronal cell monolayer was stabilized by γ-irradiation (5000 Rads.) or when these cells were eliminated almost entirely by addition of 5×10^{-6}M cytosine arabinoside to the culture medium.

Nearly all of the spinal cord neurons that survive in vitro are extremely sensitive to iontophoretically applied GABA. As is the case in vivo, GABA responses were associated with an increase in membrane conductance to Cl^-. Many (but not all) of the Cl^- dependent spontaneous and stimulus evoked synaptic potentials are reversibly reduced in size by low concentrations of bicuculline and picrotoxin. Taken together these physiological and biochemical results are consistent with the notion that GABA is used as a neurotransmitter in spinal cord cell cultures. More detailed experiments at individual synapses are in progress.

It will also be important to correlate changes in function with changes in GABA metabolism. There is a 20-fold increase in high affinity GABA transport and a similar large increase in GABA synthesis during the first two weeks after plating. Accumulation of ^{3}H-GABA (synthesized from ^{3}H-glutamate) can be increased several fold by brief incubation in 5-amino-1,3-cyclohexadienylcarboxylic acid (gabaculine); a Streptomyces toxin that specifically and irreversibly inactivates GABA transaminase. (Supported by Grant NS 11160 and the Muscular Dystrophy Associations of America.)

1287 DRUG-INDUCED SHIFTS IN SYNAPTOSOME SEDIMENTATION DURING DENSITY GRADIENT CENTRIFUGATION. Joseph A. Fix* and Charles O. Rutledge. Dept. Pharmacol. and Toxicol., Sch. Pharmacy, Univ. of Kansas, Lawrence, KS 66045.

Synaptosomes from rat corpus striatum were examined for their sedimentation characteristics in sucrose density gradients after treatment of the crude synaptosomal fraction (P2) with various drugs. The aim was to test the hypothesis that an increase in the content of cytoplasmic dopamine would decrease the density of the synaptosomes by reducing osmotic water loss from the synaptosomes during centrifugation. Alterations in sedimentation characteristics were monitored by electron microscopic examination of fractions collected from the gradients, and electrochemical detection of dopamine and norepinephrine in each gradient fraction following alumina extraction and liquid chromatographic separation of the catecholamines. The major peak of dopamine observed for synaptosomes maintained in 0.32 M sucrose prior to gradient centrifugation occurs in the range of 1.05-1.10 M sucrose. In a second series of experiments the P_2 fraction was incubated in physiological buffer at 37°C with dopamine (10^{-5}M) plus pargyline (2.5×10^{-5}M). The synaptosomes were obtained by centrifugation and were resuspended in 0.32 M sucrose prior to addition to the gradient. This treatment resulted in a shift of the major dopamine peak to 0.95-1.00 M sucrose. When the experiments were repeated except that the synaptosomes were resuspended and added to the gradient in physiological buffer, there was a further shift in the major dopamine peak to 0.90-0.93 M sucrose. Electron microscopic examination of synaptosomes taken directly from the sucrose gradient fractions showed that those synaptosomes in the lighter dopamine peak were less distorted compared to dopamine synaptosomes from the heavier sucrose fractions. The synaptosomes lower in the gradient clearly occupied less volume due to their convoluted structure. The convoluted synaptosomes assumed a normal spherical shape when suspended in physiological buffer prior to electron microscopic examination. The use of drugs to alter the sedimentation of synaptosomes offers the potential of isolating synaptosomes containing primarily one neurotransmitter substance from a heterogenous synaptosomal population. Supported by USPHS NIH Grant NS 12760.

1288 GLUTAMIC ACID AND ETHANOL DEPENDENCE. William J. Freed and Elias K. Michaelis. Dept. Human Development, University of Kansas, Lawrence, KS, 66045.

The purpose of this investigation was to test the hypothesis that excess glutamate-induced neuronal excitation is responsible for the manifestations of ethanol withdrawal in animals. Male Swiss-Webster mice were made ethanol dependent by the inhalation method of Goldstein (Fed. Proc. 34: 1953, 1975), including daily injections of 68 mg/kg of pyrazole. Concentrations of ethanol in the inhalation chamber were 8.6 mg/l for the first 24 hr, 12.3 mg/l from 24-48 hr, and 13.7 mg/l from 48-72 hr. Ethanol exposure was terminated after 72 hr and seizures elicited by handling were rated on a 1 - 4 scale for at least the next 27 hr by a "blind" observer. Glutamate diethylester, a specific glutamate antagonist that produces no direct neuronal suppression of its own (cf. McLennan, Handbook of Psychopharmacology 4, p.211 Plenum, 1975) was tested for its effects on these withdrawal seizures. A dose of 480 mg/kg administered 5 hr after withdrawal decreased seizure scores by 62% over the next 3 hr, an effect that persisted for approximately 11 hr. Smaller doses, or 480 mg/kg given either at the time of removal from ethanol exposure or 2 hr thereafter, were less effective.

Behavioral activity was measured for 1 min periods in groups of 10 mice. It was found that the activity of mice undergoing ethanol withdrawal was considerably (61%) less than that of control animals. Glutamate diethylester in a dose of 480 mg/kg tended to counteract this activity decrease (by 18%, p=.064) even though this drug decreased the activity of normal controls.

To test the possibility that CNS supersensitivity to glutamate develops during ethanol dependence we administered kainic acid, a putative glutamate agonist (cf. Olney et al., Brain Res. 77: 507, 1974) to ethanol- dependent animals while these animals continued to be exposed to ethanol. A dosage level that caused convulsions in 20% of normal controls resulted in convulsions in a significantly larger (80% and 71% in two experiments) percentage of ethanol dependent animals. Chronic administration of pyrazole alone did not alter the convulsive response to kainic acid. In contrast to this result, no differences in pentylenetetrazol seizure thresholds were observed between ethanol-dependent and control animals. These findings suggest that supersensitivity to glutamate develops during ethanol dependence, and that this phenomenon contributes to the ethanol withdrawal reaction.

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1289 ELECTROCHEMICAL DETECTION OF SYNAPTOSOMAL RELEASE OF ENDOGENOUS DOPAMINE. George E. Geier*, Mark A. Dayton*, and R. Mark Wightman* (SPON: H. David Potter). Chem. Dept., Indiana University, Bloomington, IN 47401.

High performance liquid chromatography with electrochemical detection has been used to study the dynamic properties of dopamine release from synaptosomes prepared from rat corpus striatum. The sensitivity (10^{-13} mole) and the specificity for catecholamines of the detection method have made it possible to rapidly measure release of endogenous transmitter from small amounts of synaptosomal preparations (0.5mg of protein). The percentage of dopamine in each subfraction (P2, P3, P2B, etc.) has been measured, with greatest yield in the crude mitochondrial (P2) fraction. The presence of 1 mM ascorbic acid in all solutions has been found essential for reproducible dopamine measurement.

Synaptosomal release of endogenous dopamine was found to increase as a function of K^+ concentration (10 mM to 150 mM) at 37°C. Electron microscopic observations indicate the effects are not due to synaptosomal lysis. Release was found to be independent of time at incubation times greater than 2 min indicating a steady state between uptake and release is rapidly established following depolarization.

Potassium stimulated release was found to be Ca^{++} dependent. Removal of Ca^{++} caused a 70% average decrease in dopamine release, while incubation of synaptosomes with Ca^{++} ionophore A23187, (Lilly) resulted in significant increase in released dopamine.

Various concentrations of ouabain, cytocholasin B, colchicine and diamide were also used to probe the mechanism of transmitter release. Ouabain, cytocholasin B and colchicine were all found to stimulate observed release to various degrees.

These data indicate that sensitive electrochemical detection systems as first described by Adams *et al.* (Anal. Lett., 6, 465 (1973)) can be successfully used to probe rapidly occuring membrane events at levels of sensitivity not obtainable by more conventional biochemical techniques. (Supported by Research Grant PHS 5 R01 NS 08309)

1290 CSF GABA LEVELS IN INDIVIDUALS AT RISK FOR HUNTINGTON'S DISEASE. B.S. Glaeser, N.V.B. Manyam*, L. Katz* and T.A. Hare, Thomas Jefferson Univ., Philadelphia, PA and Veterans Administration Hospital, Wilmington, DE

10 ml aliquots of lumbar cerebrospinal fluid (CSF) were obtained from 10 individuals in two families each of which had a parent with Huntington's Disease (HD) as established by classical symptoms and positive family history. The ages of these at-risk individuals ranged from 11 to 21 years with a mean age of 16.4 years. Clinical examination of the 10 subjects revealed no systemic or neurological abnormalities except for a mild scoliosis in one individual. Routine clinical laboratory tests of blood, urine and the CSF specimens were within normal limits in all cases.

GABA was measured in 0.5 ml aliquots of the CSF specimens by the ion-exchange/fluorometric procedure (Glaeser and Hare, Biochem. Med. 12, 274-282, 1975). This procedure for measuring CSF GABA levels has been shown to be accurate through comparison with (1) amino acid analysis, (2) the radioreceptor GABA assay procedure (Enna et al., J. Neurochem., in press) and (3) assay by gas chromatography/mass spectrometry (Huizinga et al., N. Eng. J. Med. 296, 692, 1977).

The results of the analyses showed GABA to be present in these CSF specimens over the range of 126-315 picomoles/ml (mean ± SD 217±61). Four of these individuals had CSF GABA levels below 180 picomoles/ml, whereas the other six participants had CSF GABA levels above 230 picomoles/ml. The mean values (± SD) for these two groups were 153±22 and 260±31 picomoles/ml, respectively. Statistical analysis by the chi-square test showed that these two groups had equal variance. The Student's t-test demonstrated that the difference between the mean values of the two groups is highly significant ($P < 0.001$).

Although it is not known which of the participants will develop the symptoms of HD, it can be expected that approximately half of them have inherited the disease. In view of the reported low levels of GABA in brain and CSF of HD patients, the observed bimodal distribution of CSF GABA in presymptomatic individuals may indicate potential for this type of measurement as a presymptomatic test for HD.

1291 STRAIN-DEPENDENT VARIATIONS IN BRAIN PHENYLETHANOLAMINE N-METHYL TRANSFERASE ACTIVITY: EFFECT OF PHENYLETHANOLAMINE N-METHYL TRANSFERASE INHIBITION ON EPINEPHRINE LEVELS. Menek Goldstein, Jow Y. Lew,* Andre M. Sauter* and Yoshimi Baba.* Dept. of Psychiatry, Neurochemistry Laboratories, New York University Medical Center, New York, New York 10016.

Phenylethanolamine N-methyl transferase (PNMT) containing cell bodies have been located in two cell clusters, C_1 and C_2 cell groups of the medulla oblongata, and some PNMT containing terminals were located in the surrounding regions (T. Hokfelt et. al., Brain Research 66, 235, 1974). The PNMT activity was found to be higher in the C_1 and C_2 regions of 4 week old spontaneous hypertensive rats (SHR's) than in the corresponding Wistar Kyoto (W.K.) substrain rats (Saavadra et. al., Science 191, 483, 1976). We have now examined the PNMT activity in selected regions of the brain of Sprague Dawley (S.D.), W.K. and SHR's. In all three strains the PNMT activity is significantly higher in the C_1 and C_2 regions than in the surrounding regions. Thus, the PNMT activity is higher in the regions containing cell bodies than in the regions containing terminals. The PNMT activity in the C_1 and in the C_1 surrounding regions, as well as in the C_2 and in the C_2 surrounding regions, is significantly higher in S.D. rats than in the W.K. or SHR's.

We have also investigated the effects of a selective PNMT inhibitor, SKF 64139 (Pendleton et. al., J. Pharmacol. exp. Ther. 197, 623, 1976) on brain PNMT activity and on brain epinephrine levels. The administration of SKF 64139 (40 mg/kg, i.p.) resulted in a decrease of brain PNMT activity with a concomitant decrease in brain epinephrine levels. Three hours after the administration of the inhibitor the enzyme activity was decreased by 90% in the analyzed regions of the brain (C_1 and C_2 regions, hypothalamus). The epinephrine levels reached minimum values 3-4 hours after the administration of the inhibitor. The enzyme activity remained decreased even six hours after administration of the inhibitor and at this time period the epinephrine levels were still significantly reduced. Sixteen hours after the administration of the inhibitor the enzyme activities returned almost to control values and the epinephrine levels returned to the corresponding control values. Thus, the time course of PNMT inhibition parallels with the lowering of the the epinephrine content in the brain. These results indicate that N-methylation of norepinephrine by PNMT may be one of the rate limiting steps in the formation of epinephrine in the CNS.

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1292 ALTERATIONS IN TYROSINE HYDROXYLASE ACTIVITY IN RESPONSE TO DEXAMETHASONE IN A CELL LINE DERIVED FROM A RAT PHEOCHROMOCYTOMA. R. Goodman,* H. Herschman* and H. Thoenen (SPON: D.M. Nance). Dept. Biol. Chem., Sch. Med., UCLA, Los Angeles, CA 90024 and Biozentrum of University of Basel, CH-4056, Basel, Switzerland.

The Gl-3 cell line was established in cell culture from an experimentally induced rat pheochromocytoma tumor (S. Warren and R.N. Chute (1972) Cancer 29:327). The cells were grown on tyrosine-free RPMI 1640 medium in the presence of dexamethasone from the fifty-fourth to the seventy-first passage. After the seventh passage in this medium, the uncloned Gl-3 cell line exhibited an increased tyrosine hydroxylase specific activity when cultured in the presence of dexamethasone as compared to cells from which the dexamethasone had been removed. This effect on tyrosine hydroxylase specific activity appears to be limited to the glucocorticoid class of steroid hormones being elicited by corticosterone, dexamethasone and triamcinolone but not by other steroid hormones such as estradiol, progesterone or testosterone. Data on the time course and dose dependence will be presented as well as the effects of inhibitors of RNA and protein synthesis.

This cell line contains, in addition to the adrenergic neuronal enzyme tyrosine hydroxylase, the cholinergic enzyme choline acetyl transferase. The relationship between the influence of glucocorticoid hormones on the levels of adrenergic and cholinergic enzymes in these cells will be discussed. (This work is supported by NIH Post-doctoral Fellowship No. 1 F32 NS05586-01, by the Swiss National Foundation for Scientific Research, Grant No. 3.653.71 and the ERDA.)

1293 SYNAPTOSOMAL UPTAKE OF TRANSMITTER AMINO ACIDS IN THE HABENULAR NUCLEI. FURTHER EVIDENCE FOR A GABAERGIC PATHWAY. Zehava Gottesfeld* and David M. Jacobowitz (SPON: L. Herrenkohl). Lab. Clin. Sci., NIMH, Bethesda, MD 20014.

The high affinity uptake mechanism of various neurotransmitters by nerve-endings has been used as a sensitive tool to identify and localize transmitter-specific neurons and their pathways in the nervous system. The uptake of γ-aminobutyric acid (GABA) and glutamic acid has been studied and compared in the lateral habenula (LHb) and medial habenula (MHb) in the rat. Synaptosomes of isolated single LHb amd MHb nuclei were prepared from 300 μm fresh tissue slices sectioned by a vibratome. After 4 min of incubation at 24°C or 37°C in Krebs buffer containing $5x10^{-7}$ M ^{3}H-GABA, ^{3}H-glutamate or ^{3}H-glycine, the synaptosomes were collected by filtration, extracted and the neurotransmitters determined. High accumulation of GABA and glutamate was found in the habenular nuclei, whereas the uptake of glycine was low at this concentration. LHb and MHb showed a differential uptake capacity for ^{3}H-GABA and ^{3}H-glutamate. The LHb accumulated twice as much GABA and 3 times as much ^{3}H-glutamate compared to MHb. The uptake was shown to be sodium-dependent and was abolished in the presence of 0.1% Triton X-100 or hypotonic conditions. The accumulation of ^{3}H-GABA by the LHb was not affected by 10^{-5} M amino-oxyacetic acid, a GABA-transaminase inhibitor. L-2,4-diaminobutyric acid (1 mM), a potent inhibitor of GABA uptake by nerve endings, and β-alanine (0.5 mM), an inhibitor of GABA uptake in glial cells, caused a reduced uptake of ^{3}H-GABA in the LHb by 70% and 30%, respectively.

High-frequency stereotaxic lesions placed in the stria medullaris (SM), a major pathway which connects various brain regions with the habenular nuclei, resulted in a 41% and 62% decreased uptake, respectively, of ^{3}H-GABA in the LHb after 4 days or 5 weeks survival. No change was observed in the MHb.

The differential uptake of the transmitters by the LHb and MHb may indicate a higher concentration of both GABAergic and glutamate-containing terminals in the LHb than the MHb. In addition, the SM lesion-induced fall in GABA uptake is proportional to the loss of glutamic acid decarboxylase, a GABA-synthesizing enzyme, observed in the LHb (Gottesfeld et al., Brain Res., 1977, in press). This provides further evidence for possible GABAergic afferents via the SM to the LHb.

1294 GABA MEASUREMENT IN HUMAN CEREBROSPINAL FLUID: BASIC CONSIDERATIONS. M.H. Grossman*, T.A. Hare, W.W. Tourtellotte*, J.L. Alderman, L. Katz* and N.V.B. Manyam*. Thomas Jefferson University, Philadelphia, PA, V.A. Wadsworth Hospital, Los Angeles, CA and V.A. Hospital, Wilmington, DE

Parameters relevant to the clinical significance of cerebrospinal fluid (CSF) GABA measurements were studied. GABA was measured using the ion-exchange/fluorometric procedure (Glaeser and Hare, Biochem. Med. 12, 274-282, 1975).

The stability of GABA in CSF under various conditions of collection and storage was studied using lumbar specimens which were drawn and then, as quickly as possible, mixed and divided into equal aliquots. One of these aliquots was immediately frozen in dry ice and subsequently stored at -70°C. A second aliquot was also immediately frozen in dry ice but then maintained in storage at -20°C. A third aliquot was allowed to stand at room temperature for 10 min, then frozen in dry ice and stored at -70°C, and a fourth aliquot was allowed to stand at room temperature for 120 min then frozen in dry ice and stored at -70°C. The specimens were stored for 16 months before analysis. The GABA levels determined for the second, third and fourth aliquots were divided by that determined for the first aliquot to provide a ratio indicative of the change of GABA level. The data indicated that storage at -20°C was nearly equivalent to storage at -70°C (ratio = 1.12). Maintaining the samples at room temperature for 10 min did not significantly influence the GABA level (ratio = 1.01); whereas, maintaining the samples at room temperature for 120 min resulted in an increase of GABA concentration (ratio = 1.97).

GABA was also measured in lumbar CSF from 5 normal pressure hydrocephalus patients which gave a mean (± SD) value of 124±56 picomoles/ml, and in ventricular CSF from two other normal pressure hydrocephalus patients which gave values of 301 and 250 picomoles/ml.

In addition, aliquots of lumbar CSF specimens from 12 neurologically normal individuals were obtained and analyzed for their GABA content. The mean volume drawn was 19±4 ml. The mean age of the subjects was 24±3 yrs. The mean GABA level was 515±160(SD) picomoles/ml.

1295 HYPERPOLARIZING RESPONSES TO HISTAMINE (HA) IN APLYSIA NEURONS: IONIC MECHANISM AND PHARMACOLOGY OF THE HA RECEPTORS.
D.L. Gruol* and D. Weinreich* (SPON: N. Brookes). Univ. Md. Sch. Med., Dept. Pharm., Baltimore, MD. 21201.

In the cerebral ganglia of the marine mollusk, Aplysia californica, an identifiable group of neurons are sensitive to iontophoretically applied HA, a putative neurotransmitter in the vertebrate and invertebrate CNS. We have utilized electrophysiological techniques to study the ionic mechanism of the HA responses and the pharmacology of the HA receptors in these neurons. When HA was applied iontophoretically to the soma or axon hillock region of the HA sensitive neurons the most common response was a slow hyperpolarization (10-20 sec) mediated by an increased K^+ conductance. Less frequently the response was biphasic and consisted of a fast hyperpolarization ($\sim$ 2 sec), mediated by an increased Cl^- conductance, superimposed on a slow hyperpolarization identical to that described above. The slow hyperpolarization was mimicked by several HA analogues, the most potent being the H_1-agonist 2-MeHA and the H_2-agonist 4-MeHA. None of the 15 HA analogues or metabolites tested mimicked the HA fast response. The HA sensitive neurons also showed K^+ and Cl^- conductance increases to iontophoretically applied ACh. The ACh responses and anticholinergic drugs were used to evaluate the selectivity of the HA receptor antagonists. The slow HA response elicited by HA or HA agonists could be completely, selectively and reversibly blocked by bath application of the H_2-antagonist cimetidine. The other H_2-antagonists, burimamide and metiamide, gave variable results and were often non-selective. The H_1-antagonists were either ineffective (diphenylpyraline, chloropheniramine) or partially blocked (pyrilamine) the response in a non-selective manner. The fast HA response was not blocked by any of the H_1- or H_2-antagonists tested, but was completely suppressed by curare or strychnine, drugs which are thought to act on the Cl^- iontophore. These results suggest that two pharmacologically distinct receptors mediate the K^+ and Cl^- HA responses in Aplysia neurons. Neither type of receptor could be classified as H_1 or H_2 according to the criteria presently used in other systems. The selectivity and reversibility of cimetidine indicate that this antagonist should be a valuable pharmacological tool for defining putative histaminergic synapses in Aplysia and other nervous systems.
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1296 INCREASE IN RAT CAUDATE NUCLEUS CHOLINE ACETYLTRANSFERASE ACTIVITY FOLLOWING CHOLINE ADMINISTRATION VIA STOMACH TUBE.
M. J. Hirsch and R. J. Wurtman. Lab. Neuroendocrine Reg., MIT, Cambridge, MA 02139.

Choline chloride (ChCl) administration, by injection (Life Sci. 16:1095, 1975), stomach tube (Neurosci. Abstr. 2:765, 1976), or via the diet (Science 191:561, 1976), elevates blood choline, brain choline, and brain acetylcholine (ACh) concentrations in the rat. Prolonged increases in both brain compounds, lasting at least 8 hours, are observed when choline is administered by stomach tube. We have utilized this technique for elevating brain ACh levels to determine whether such elevations are associated with feedback changes in the activity of choline acetyltransferase (CAT), the enzyme that catalyzes the synthesis of ACh from choline and acetyl coenzyme A.

Among normal, unfasted, 200-g rats intubated with 2.8 g/kg ChCl and killed by decapitation 8, 24, or 48 hours later, CAT activity was significantly elevated 8 hours after ChCl, and remained elevated after 24 hours. Both choline and ACh levels were elevated 8 hours after ChCl, but did not differ from control levels 24 hours later. No changes in CAT activity, choline, or ACh content were observed 48 hours after ChCl.

Hours after ChCl	CAT activity (nmols ACh formed per hr/mg tissue)		Choline % of control	ACh % of control
	Control	ChCl-treated		
8	5.11 ± 0.35	6.37 ± 0.46*	329†	136‡
24	7.35 ± 0.57	8.77 ± 0.62†	108	88
48	9.19 ± 0.73	9.29 ± 0.77	108	95

*P < 0.02, †P < 0.001, ‡P < 0.05, compared with control.

These data indicate the lack of negative feedback control of CAT activity in vivo when ACh levels are increased; no reduction in CAT activity is observed either after brief periods (e.g., as could be caused by allosteric changes or by end product inhibition) or after times that might be compatible with depressed enzyme synthesis. Moreover, CAT activity increases significantly after choline, suggesting that positive feedback mechanisms (perhaps related to excitatory collateral cross-innervation) may operate.
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1297 A MAPPING OF THE DISTRIBUTION OF ACETYLCHOLINE, CHOLINE ACETYLTRANSFERASE, AND ACETYLCHOLINESTERASE IN DISCRETE AREAS OF THE RAT FOREBRAIN.
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Previous biochemical studies of the distribution of cholinergic markers in rat brain have generally dealt only with acetylcholine (ACh) and choline acetyltransferase (CAT). Because of the wealth of histochemical information available about the location of acetylcholinesterase (AChE)-containing neurons, we have also included this enzyme in the present mapping of cholinergic parameters in the rat forebrain. Male Sprague-Dawley rats were killed by 2.5-3.0 seconds of microwave irradiation of the head region prior to ACh determinations and by decapitation for enzyme assays. Coronal sections (300 µm) of microwave-fixed brains were cut in a vibratome using an inert fluorocarbon bath to prevent diffusion. Brains from decapitated rats were sectioned in a cryostat. Regions were dissected by a micropunch procedure and assayed for ACh, CAT, and AChE by sensitive radiometric methods. ACh, CAT, and AChE were distributed throughout the rat forebrain. Approximately a 5-fold difference was found in ACh levels between the regions with the highest and those with the lowest concentrations (see table below). The ranges for CAT and AChE were larger. ACh was found in a number of forebrain regions not previously examined. Many areas had ACh levels higher than those found in the hippocampus, a region with a well defined cholinergic input. We feel that this emphasizes the probable importance of ACh in a number of forebrain functions. A good correlation was usually found between the distribution of CAT and AChE. This information may be of value in interpreting AChE-histochemistry. The cholinergic enzymes showed less correlation with ACh levels. Such biochemical measurements should prove valuable in experimental manipulations designed to localize cholinergic pathways and in the study of drug effects of ACh concentration in discrete regions of the brain.

Region	ACh(pm/µgP)	CAT(pm/µgP/hr)	AChE(nm/µgP/hr)
N. caudatus putamen	0.89 ± 0.08	184 ± 11	25.0 ± 1.6
Globus pallidus	0.44 ± 0.04	28.5 ± 5.9	5.01 ± 0.26
N. preopticus medialis	0.26 ± 0.02	9.4 ± 1.5	3.18 ± 0.20
N. tractus diagonalis	0.59 ± 0.05	249 ± 25	25.8 ± 5.2
Dorsal hippocampus	0.25 ± 0.03	48.1 ± 2.1	3.54 ± 0.17
Parietal cortex	0.16 ± 0.01	32.2 ± 1.7	2.49 ± 0.09
Piriform cortex	0.45 ± 0.05	91.8 ± 5.5	6.54 ± 0.57
Median eminence	0.23 ± 0.02	126 ± 9	2.66 ± 0.18
N. ventromedialis	0.31 ± 0.03	26.5 ± 4.7	5.59 ± 0.56
N. amygdaloideus cent.	0.45 ± 0.05	50.0 ± 3.9	4.28 ± 0.26

1298 RELEASE OF ENDOGENOUS NOREPINEPHRINE AND DOPAMINE FROM RAT BRAIN REGIONS IN VITRO.
G. Jean Kant and James L. Meyerhoff, Division of Neuropsychiatry, Dept. Med. Neurosciences, Walter Reed Army Inst. of Research, Washington, D.C. 20012

Using sensitive radioenzymatic assay procedures, it is now possible to measure the picomolar amounts of endogenous norepinephrine (NE) and dopamine (DA) released in vitro. We have previously measured the release of NE in vitro from hypothalamus, a region with a high NE concentration, in response to depolarizing concentrations of KCl, (Kant and Meyerhoff, Life Sciences, 20 (1977) 149-154). We also reported that DA was released from striatum, a region rich in DA, after incubation with KCl or d-amphetamine, (Balcom and Meyerhoff, Neuroscience Abstracts, 2 (1976) 773). We have now extended our investigation to other regions of rat brain that have much lower catecholamine levels. We find detectable release of NE and DA in response to KCl or 10^{-4}M d-amphetamine in many regions of brain.

The release of NE and DA in response to KCl stimulation was examined in 6 regions: cortex, hippocampus, hypothalamus, striatum, combined accumbens-olfactory tubercle, and substantia nigra. Tissue pieces were dissected, weighed, chopped (.3 mm^2), and washed in cold Krebs-bicarbonate buffer. The tissue was then resuspended in 1.5 ml of warm buffer with or without additional KCl (50mM) and incubated for 5 min. at 37°C. Release was terminated by pouring the tissue suspension through a millipore filter. Filtrates were assayed for NE and DA. NE release in response to KCl was detectable in all regions except striatum. Amounts released after KCl (expressed as % control) were cortex (313%), hippocampus (227%), hypothalamus (225%), accumbens-tubercle (278%), s.nigra (155%). KCl stimulated release of DA was detected in 3 regions: striatum (414%), accumbens-tubercle (282%), and hypothalamus (312%). DA was measurable in filtrates from the s. nigra but levels from control and KCl stimulated samples were equal. DA release was not detectable from cortex or hippocampus.

The release of NE and DA after incubation with 10^{-4}M d-amphetamine was measured in 11 regions of rat brain: cortex, hippocampus, hypothalamus, striatum, n.accumbens-olfactory tubercle, s.nigra, septal region, olfactory bulb, cerebellum, brainstem, and midbrain. Tissue pieces were dissected, chopped and washed in Krebs-bicarbonate prior to resuspension in 1.0 ml of warm buffer and incubation for 10 minutes with or without 10^{-4}M d-amphetamine. Release was terminated by centrifugation and the supernatants were assayed for NE and DA. d-Amphetamine stimulated NE outflow compared to control in all regions examined. DA release was markedly increased in most regions especially striatum (287%), hypothalamus (387%) and accumbens-tubercle (670%).

1299 BLOOD HISTAMINE LEVELS IN SCHIZOPHRENICS AND NORMAL CONTROLS. R.-L. Lin* N. Narasimhachari, Carl O. Kinard* and J. M. Davis. Department of Research, Illinois State Psychiatric Institute, Chicago, Illinois 60612.

The rarity of allergic diseases in schizophrenics has probably been attributed to an unusual histamine resistance. An increase of reactivity to intradermal histamine has also been found when schizophrenics improved under ECT. An increased concentration of histamine in the plasma or serum of schizophrenics was reported by earlier investigators. More recently, Pfeiffer et al[1] determined blood histamine by the fluorometric method with OPT and reported that a schizophrenic outpatients population has been found to be 50% histapenic and 20% histadelic. Members of the low histamine group have paranoia and hallucinations, while those of high group have suicidal depression.

A specific radio-enzymatic assay of histamine has been described by Kobayashi and Maudsley[2] and by Taylor and Snyder.[3] We have modified this specific method using partially purified histamine-N-methyltransferase from pork kidney as enzyme source, and high specific activity of S-adenosylmethionine (methyl ^{3}H). This enzyme preparation showed no activity of other methyltransferases such as indolethylamine-N-methyltransferase, catechol-O-methyltransferase, methanol-forming enzyme, etc. which utilize S-adenosylmethionine as methyl donor. We found that dithiothreotol enhanced histamine-N-methyltransferase activity, therefore, this thio-reagent was added to our assay system. Instead of extracting the reaction product with organic solvents, we used charcoal absorption method which has been developed for choline acetyltransferase assay by Wu et al.[4] The assay is linear from 0.5 ng to 10 ng of histamine and 1 ng of histamine gave 6,000 cpm above the assay blank. With this modified radio-enzymatic assay, we have studied the blood histamine levels of schizophrenic patients and normal controls. This study is carried out double blind with patients and normal controls. The data obtained after breaking the code of samples will be presented and the significance discussed.

1. Rev. Can. Biol., 31, 73 (1972).
2. Anal. Biochem., 46, 85 (1972).
3. J. Neurochem., 19, 1343 (1972).
4. Trans. Amer. Soc. Neurochem., 8, 169 (1977).

1300 PHARMACOLOGY OF ^{3}H-GABA BINDING TO HUMAN CEREBELLAR CORTEX. Kenneth G. Lloyd and Syd Dreksler* Clarke Institute of Psychiatry Univ. of Toronto, Toronto, Ont., Canada, M5T 1R8.

The high affinity (K_D= 0.33 μM) sodium-independent binding of ^{3}H-GABA to human cerebellar cortex and its state in pathological conditions (Parkinson's disease and Huntington's chorea) has previously been reported by us and others. In the rat brain, the pharmacology of ^{3}H-GABA binding has been studied by Enna and Snyder (Br.Res. 100,81,1975) and has been proposed to be closely associated with the GABA receptor. We have examined the effects of various drugs on the ^{3}H-GABA binding to membranes prepared from human cerebellar cortex, by methods previously described (Lloyd et al, Br.Res.,In Press). The ID50's for ^{3}H-GABA binding of the active compounds are presented in the Table. These results are strongly supportive for the hypothesis that the ^{3}H-GABA binding in the human cerebellar cortex is to GABA receptors and that the human GABA receptor has a pharmacological profile similar to, but not identical with, that of rat brain. Thus, the relative affinities of the active drugs parallel those seen for the reversal of bicuculline on spinal interneurons (Curtis et al, Br.Res. 32,69,1971) and on the displacement of ^{3}H-GABA binding to membranes from rat brain (Enna and Snyder). The inactivity of β-alanine, DABA, AOAA and dipropylacetate indicate that the ^{3}H-GABA binding observed is not associated with neuronal or glial GABA uptake mechanisms or with the enzyme GABA transaminase. The results indicate that ^{3}H-GABA binding is a valid procedure for examining the state of GABA receptors in pathological conditions and also for the study of drug affinities to GABA receptors.

Table. ID50's for the Displacement of ^{3}H-GABA from Human Cerebellar Membranes.

Active Compounds			
ID50 0.01-0.1 μM	ID50 0.1-1.0 μM	ID50 1.0-10 μM	ID50 10-100 μM
Muscimol	GABA	Imidazole Acetic A.	Bicuculline
		δ-Aminovaleric A.	Strychnine
		β-OH-GABA	d-Tubucurare
		Homocarnosine	

Inactive Compounds (less than 50% displacement at 10^{-3}M)		
β-Alanine	Baclofen	Premarin
Procaine	Dipropylacetate	Glycine
Glutamate	Picrotoxin	Taurine
γ-Hydroxybutyrate	AOAA	Diazepam
Propoxyphene	DABA	Atropine

1301 REGIONAL DISTRIBUTION OF NEUROTRANSMITTER CANDIDATES IN THE CNS OF THE MOTH MANDUCA SEXTA, WITH SPECIAL REFERENCE TO THE VISUAL SYSTEM Gerald D. Maxwell and Jonathan F. Tait* Department of Neurobiology, Harvard Medical School, Boston, Mass. 02115

Using the radiochemical screening procedure of Hildebrand et al. (J. Neurobiol. 2:231-246, 1971) we have assayed several regions of the CNS of Manduca for their ability to synthesize acetylcholine (ACh), γ-aminobutyric acid (GABA), norepinephrine (NE), dopamine (DA), octopamine (OA), 5-hydroxytryptamine (5HT), tyramine (TA), and histamine (HA). Animals were used 2-3 days prior to adult eclosion. Brains, first thoracic, third abdominal, and fourth abdominal ganglia were removed from the animal and incubated separately in 50 μl of modified Grace's insect tissue culture medium containing the appropriate high specific activity tritium-labeled precursor compound at a concentration of 50-100 μM. After incubation the brain was dissected into antennal lobes, optic lobes, protocerebrum minus optic lobes, and subesophageal ganglion. The brain fragments and segmental ganglia were then homogenized in pH 1.9 formate-acetate buffer and the supernatant electrophoresed to separate the precursor and product. All neurotransmitter candidates were synthesized in at least some of the structures, except NE, which was not reliably detected in any of the structures. The absolute amount of neurotransmitter synthesis was greatest in the case of ACh. In particular, the ACh pool was highly labeled in the antennal lobes, which are known to contain high endogenous levels of ACh and choline acetyltransferase from the work of Prescott et al. (Comp. Bioch. Physiol. 56C:77-84, 1977). ACh was also synthesized in the optic lobes where it is probably associated with interneurons. A striking distribution of 5HT synthesis occurred; it was highly labeled in the optic lobes and abdominal ganglia, and negligibly in other brain areas, when synthesis is compared on a per gram protein basis. Further work has shown that 5HT synthesis is about five times greater in a fragment containing the lamina (first synaptic neuropil) and part of the retina containing photoreceptor cell bodies, than in a more proximal fragment containing the higher order synaptic neuropils. When the distribution of other neurotransmitter candidates found in the visual system was examined in this manner it was found that the distribution of synthesis of ACh, GABA, and HA was nearly equal in the proximal and distal fragments of the visual system. Experiments involving the incubation of fragments of retinal tissue indicate that substantial amounts of HA and GABA may be made in the retina. (Supported by NIH grant NS-11010 to Dr. John G. Hildebrand and postdoctoral fellowships from NIH and the Muscular Dystrophy Association to GDM).

1302 GLYCINE UPTAKE BY THE APLYSIA PARIETO-VISCERAL GANGLION NEURONS R3-R14. D.J. McAdoo, T.M. Iliffe*, C.H. Price and R.A. Novak*. Mar. Biomed. Inst. and Dept. HBC&G, Univ. of Texas Med. Branch, Galveston, TX 77550.

Endogenous glycine concentrations in R3-R14 are 5-20 times higher than in other Aplysia neurons[1], and R3-R14 were more intensely labeled than their neighbors in autoradiograms of sections of ganglia incubated in ^{3}H-glycine-containing sea water. Therefore, glycine uptake by R3-R14 and other identified Aplysia abdominal ganglion neurons was characterized by analyses performed on individual cell bodies dissected from ganglia incubated in artificial sea water containing labeled glycine. Glycine was taken up by R3-R14 twice as rapidly as by other Aplysia neurons, while alanine, serine and leucine were each taken up at the same rate by R3-R14 as by other Aplysia neurons. Less than 5% of the glycine taken up by R3-R14 and most other Aplysia neurons was incorporated into protein after a 1 hr incubation, though nearly half of the radioactivity in the neurosecretory bag cells was macromolecular. Some glycine was transformed into serine in all examined neurons, with the reverse reaction occurring to a lesser degree. The uptake of glycine into neurons other than R3-R14 was not greatly affected by the absence of Na^+ or the presence of Hg^{++}, while these conditions caused glycine uptake by R3-R14 to become indistinct from that of the other neurons. We therefore conclude that there is a general glycine uptake system common to all Aplysia neurons, and an additional specific one associated with R3-R14. Dinitrophenol and ouabain had little effect on glycine uptake by R3-R14 after 5 min, indicating that though glycine uptake by R3-R14 is driven by the Na^+ gradient across the cell membranes, it is not directly coupled to the activity of Na^+/K^+ ATPase. Glycine uptake by both uptake systems became saturated at about a 1 mM external glycine concentration, so both uptake systems are carrier mediated. The glycine-specific uptake system associated with R3-R14, together with the high endogenous glycine concentrations in those neurons, indicates that glycine must have a specific function in R3-R14, possibly that of a neurotransmitter.

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[1]T.M. Iliffe, D.J. McAdoo, C.B. Beyer and B. Haber, J. Neurochem., May, 1977.

1303 ADENOSINE-DOPAMINE INTERACTIONS IN CAUDATE SYNAPTOSOMES. Mary Michaelis, Sharie Myers*, David Goering*, and Elias Michaelis. Dept. Human Development, U. of Kansas, Lawrence, KS. 66045

The purine nucleoside adenosine (Ado) is now believed by many investigators to be a significant modulator of neuronal activity in the CNS as well as in the periphery. Adenosine's effects on c-AMP generating systems has been studied extensively as has its physiological activity in cortical neurons. This nucleoside has also been demonstrated to be transported in both retrograde and anterograde directions and to be released upon electrical stimulation. However, the exact nature of the role(s) that Ado plays in modulation of neuronal processes has yet to be fully characterized. As an approximation to further characterization of this nucleoside's influence, we have examined two possible sites of interaction between Ado and dopamine (DA) in a crude synaptosomal preparation from the rat caudate nucleus. In addition, we have examined the ability of Ado to stimulate the adenylate cyclase activity of a broken cell preparation from the rat striatum.

One of the most frequently observed actions of Ado and its analogs is an inhibition of release of neurotransmitters. Consequently, the effects of 2-chloroadenosine (2-Cl-Ado), a less readily metabolized analog of Ado, on KCl-stimulated release of DA from caudate synaptosomes have been studied by means of Millipore filtration. Synaptosomes were pre-loaded with ^{3}H-DA and release of ^{3}H-metabolites was determined after 30 sec incubation of 37° in the presence of 15mM KCl. The addition of 2-Cl-Ado (.066-6.6μM) showed a dose related inhibition of release of ^{3}H-DA and its metabolites with a maximum of 25% inhibition. This effect was greatly reduced when 50μM caffeine, an Ado antagonist, was present in the medium. In addition, synaptosomes preincubated with adenosine deaminase for 60 sec prior to addition of KCl showed an enhanced release of ^{3}H-compounds (25-30%), suggesting that more rapid termination of the activity of endogenous Ado allows for greater release of DA.

It has been proposed that Ado formation in brain proceeds from ATP through a Ca^{++}-dependent ATPase, an adenylate kinase, and finally a 5'-nucleotidase. In both spectrophotometric and radioassay methods we have found this synthetic pathway for Ado in striatal synaptosomes to be partially inhibited (15-35%) by DA in the concentration range of 0.25-5μM. These observations, in addition to our demonstration that 2-Cl-Ado and Ado itself in micromolar concentrations are potent stimulators of striatal adenylate cyclase (300-600% stimulation), suggest that Ado and DA may participate in some reciprocal interactions which could serve to modulate the final output from some striatal neurons. (Supported by DHEW Service Award HD07066 and by grant AA01911)

1304 LEVELS OF NOREPINEPHRINE IN PLASMA OF SYNCOPAL SUBJECTS. E.J. Mikkelsen*, C.R. Lake, M.G. Ziegler*#, G.L. Brown*, M.H. Ebert*. NIMH, Bethesda, MD 20014. #Univ. of Texas Med. Br., Galveston,TX

The sympathetic nervous system (SNS) is responsible for the maintenance of blood pressure upon assuming upright posture. Norepinephrine (NE) is the primary neurotransmitter of the SNS and is normally released upon standing, thus increasing peripheral resistance and maintaining blood pressure and cerebral perfusion. There are multiple physiological and psychic factors which predispose to fainting in individuals without a specific SNS defect.

In a normal population of healthy volunteers about 12% became faint with symptoms of yawning, nausea, pallor, and sweating when blood was drawn with the subjects in a standing position. Only about 4% lost consciousness. The mean (±SEM) age of the syncopal subjects was 22±2 years which was significantly ($p<0.001$) less than that of controls (36±2) who did not feel faint. Basal (resting, supine) levels of NE in 25 subjects (17 males and 8 females) who subsequently became faint after standing was 350±33pg/ml which was greater than that (281±17) of 64 control subjects of the same mean age. Basal pulse rate (P) in the fainters was also significantly higher ($p<0.005$) than in the controls (85±3 vs. 74±1 respectively). Basal blood pressures (BP) in the fainters and controls were $\frac{116\pm2}{73\pm3}$ and $\frac{112\pm2}{69\pm1}$ respectively. Blood was drawn from 12 subjects during a syncopal attack but before the subjects either sat down or lost consciousness. P was significantly elevated in these 12 dizzy standing subjects when compared to 51 comfortably standing subjects but their levels of NE (564±91) were not significantly greater than the control's value of 517±31. Concomitantly, BP was lower in the standing syncopal subjects ($\frac{93\pm4}{69\pm4}$) than in the controls ($\frac{109\pm2}{75\pm1}$).

A possible explanation for the syncope upon standing is a diminished release of NE from sympathetic nerve endings. The % increase of NE in the fainters after standing at least 5 minutes was 42±9% which is lower ($p<0.001$) than the % increase in the controls of 88±8%. P ($p<0.01$) and diastolic BP ($p<0.025$) increased more after standing in the controls (26±3% and 12±3%) than in the syncopal subjects (12±5% and 0.6±4%). Systeolic BP fell more ($p<0.02$) in the fainters (20±3%) vs. 0.7±2% in the controls after standing.

Bradycardia secondary to vagal discharge (vaso-vagal syncope) is well known. Of our 12 subjects that we measured P and NE while standing and symptomatic, 3 had pulse rates of 80 beats/min. or less, the other 9 had P over 100; NE levels were low in only one of these patients. Thus vagal discharge may have occured in 25% of our syncopal subjects and plasma levels of NE were not related to the vagal discharge.

1305 EFFECT OF DEPOLARIZING AGENTS ON THE PERCENT SATURATION OF GLUTAMATE DECARBOXYLASE (GAD) WITH PYRIDOXAL-5'-PHOSPHATE (PLP) IN RAT SUBSTANTIA NIGRA SYNAPTOSOMES. L.P.Miller*& J.R.Walters. NIH, NINCDS, Bethesda, MD. 20014.

Recent observations have shown that GAD from rat whole brain is only 35% saturated with cofactor, PLP, in vivo (Nature 266: 847, 1977). These studies also indicated the importance of using appropriate preparative procedures for investigation of the in vivo interaction of GAD with cofactor, since cofactor-mediated activation of the enzyme occurs in situ following decapitation and in vitro during homogenization. It was proposed that the low level of saturation of GAD by PLP reflects a dynamic balance between the rate of dissociation of the cofactor from the enzyme, caused by glutamate, and the rate of association of the cofactor with the apoenzyme, which is inhibited strongly by the nucleotides, ATP and ADP.

To confirm these observations and to investigate the possibility that conditions thought to increase transmitter release and turnover might be associated with alterations in the amount of GAD associated with PLP, synaptosomes were prepared from rat substantia nigra punches and incubated in Krebs-Ringer buffer. After varying periods of incubation, aliquots of the synaptosomal suspension were taken for determination of the degree saturation of GAD by cofactor. The synaptosomes were homogenized in imidazole buffer containing Triton X-100(0.5%) and ATP(5mM),included to prevent activation of the enzyme by endogenous PLP during homogenization. The homogenate was passed through a Sephadex G-25 column and analyzed for GAD activity in the presence and absence of PLP. The degree saturation of GAD was defined as enzyme activity in the absence of PLP, expressed as a % of the activity obtained in the presence of saturating PLP(50um). GAD from unincubated synaptosomes was 29% saturated with cofactor, confirming previous observations that the saturation of GAD by cofactor in vivo appears to be quite low. After 5 min of incubation at 37°C, the degree saturation decreased to 19% and remained relatively constant for 30 min. In the presence of a depolarizing concentration of K^+ (55mM), the % saturation became greater with time; a 48% increase was observed at 20 min. When veratridine was used as a depolarizing agent, this effect was even greater. The increase in the amount of GAD saturated by PLP was dependent on Ca^{++}. In the absence of Ca^{++}, the % saturation was lower than that observed in the non-K^+-stimulated controls. If conditions existing during synaptosomal depolarization can be considered comparable to those of increased transmitter release in vivo, then these results suggest that in GABA neurons increased neuronal activity may be associated with an increase in the amount of functional, PLP-saturated GAD, which, in turn, could cause a potentiation of the rate of GABA synthesis.

1306 THE RELEASE OF IMMUNOREACTIVE SUBSTANCE P AND SOMATOSTATIN FROM SENSORY NEURONS IN DISSOCIATED CELL CULTURES. Anne W. Mudge*, G.D. Fischbach, and S.E. Leeman. Departments of Physiology and Pharmacology, Harvard Medical School, Boston, Mass. 02115

Substance P and somatostatin have been shown by immunological techniques to be present in some dorsal root neurons and in their processes which terminate in the dorsal horn of the spinal cord. Considerable evidence indicates both peptides may be involved in synaptic transmission at this site and other regions of the nervous system. Cell culture provides an opportunity to study these peptides in identified populations of neurons where the neurons are easily accessible to biochemical and electrophysiological manipulations. The interpretation of biochemical data is clearer when neurons are grown in the virtual absence of nonneuronal cells.

Sensory neurons were dissociated from dorsal root ganglia of 9-day chick embryos and grown on collagen in medium containing nerve growth factor. Addition of cytosine arabinoside for three days resulted in cultures essentially free of nonneuronal cells. The surviving neurons (~20%) grew in size and developed a dense network of processes. Immunoassayable substance P increased from 100 fmol at the time of plating to 2500 fmol per dish after two weeks in culture. Immunoassayable somatostatin was approximately 250 fmol per dish at two weeks.

The cultures were incubated in HEPES-buffered balanced salt solutions containing either (a) Na^+,113mM; K^+,5.9mM; Ca^{++},1.8mM; Mg^{++}, 0.8mM or (b) Na^+,17mM; K^+119mM; Ca^{++}, 1.8mM; Mg^{++}0.8mM. During five minutes in control solution (a) the neurons released 0.35% of their substance P content; the release of somatostation was undetectable (<1.5%). When incubated for five minutes in depolarising concentrations of K^+ (b), the neurons released 4.3± 0.4% (mean ± SEM of 9 observations) of their substance P and 6.9 ±0.4% (5 observations) of their somatostatin. Repeated exposure to high K^+ solutions resulted in release of similar amounts of both peptides. There was no difference in the release of 2-^{14}C-deoxyglucose, a cytoplasmic marker, after incubation in either solution. The K^+-evoked release could be reversibly blocked by 5mM Co^{++}, a competitive inhibitor of Ca^{++} fluxes. Thus primary sensory neurons in vitro can make both substance P and somatostatin and release them in a voltage - and Ca^{++}-dependent manner.

1307 ACTIVATION OF SYNAPTOSOMAL TRANSPORT SYSTEMS BY DEPOLARIZATION. L. Charles Murrin*, Mary S. Lewis*, Nikolai Klemm* and Michael J. Kuhar, Dept. Pharmacol & Exp. Ther., Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21205.

The synaptosomal high affinity transport of choline has been shown, by in vivo and in vitro experiments, to be coupled to neuronal activity; i.e., uptake is increased by increased neuronal activity in vivo and by depolarization of synaptosomes in vitro. In this study we report the effects of high potassium depolarization on synaptosomal transport of a number of compounds including adenine, adenosine, alanine, arginine, aspartic acid, choline, glutamic acid, GABA, glycine, leucine, octopamine, ornithine, phenylalanine, proline, serine, serotonin, taurine, tryptophan and tyrosine. A crude mitochondrial preparation (P_2) from rat cerebral cortex was preincubated in either normal Krebs Ringer phosphate (KRP) or 65mM K^+-KRP at 37° for 10 min. This synaptosomal preparation was centrifuged, the pellets resuspended in sucrose and used in uptake experiments (Molec. Pharmacol. 12:1082). Uptakes were carried out in KRP for 4 min. at 37° with 4° blanks. All compounds were used at a concentration of 1 μM. Depolarization increased the synaptosomal transport of several compounds which are known or suspected neurotransmitters or neurotransmitter precursors, including choline, glutamic acid, aspartic acid, glycine and phenylalanine. This was not a universal property of neurotransmitter precursors since the transport of tyrosine and tryptophan did not increase after depolarization. Similarly, the transport of many other compounds was not increased by depolarization. These include serine, taurine, proline, leucine, alanine, adenine, adenosine, arginine, ornithine, GABA and serotonin. The depolarization-induced increases in transport were found, by sucrose density centrifugation, to be associated with the synaptosomal fraction and were found to be eliminated by hypo-osmotic shock. They were also found to be dependent on membrane potential. Finally, kinetic analyses of these transport alterations were carried out.

In conclusion, it appears that depolarization of synaptosomes, which mimics increased neuronal activity, produces an increase in transport of several compounds. These in vitro data lead us to propose that certain transport systems in nerve terminals are coupled to neuronal activity, presumably for regulating neurotransmitter stores or other metabolic functions. Supported by MH 25951.

1308 AMPHIBIAN OPTIC NERVE TRANSMITTER: ACh, YES; GABA AND GLUTAMATE, NO. Robert E. Oswald* and John A. Freeman. Vanderbilt University, Nashville, TN 37232.

The amphibian retinotectal system provides a useful model for the study of molecular events associated with synaptogenesis in the central nervous system. Previous studies using labeled snake α-neurotoxins have indicated (Freeman, Nature 1977, in press) that the maintenance of retinotectal synapses in the toad B. marinus is associated with the functional integrity of the tectal nicotinic cholinergic receptor protein. The present study was undertaken to establish the identity of optic nerve neurotransmitters in this animal. Synaptosomal fractions from whole tectal homogenates were screened for high affinity uptake systems for several different possible transmitters. A saturable Na^+-dependent high affinity uptake system was present for choline ($Km=2.3x10^{-6}$ M), and also for GABA and glutamate. Significant levels of choline acetyltransferase were found in both tectum and optic nerve (192 pM and 15 pM ACh synthesized/mg protein/minute, respectively). A high tectal ACh level (61±3 nmoles/gm tissue) was also found, using pyrolysis gas chromatography-mass spectrometry on microwave inactivated tissue. Tectal synaptosomes bound α-bungarotoxin (Kd=10.78 nM); saturation of the specific toxin binding component occurred at 4.2 pmoles toxin/gm tissue. No decline in the high affinity uptake for either choline or glutamate occurred within 60 days following enucleation, although there was a significant fall in the levels of both choline acetyltransferase and ACh. The interpretation of these results should take into account the effects of any gliosis or sprouting of non-retinal terminals that might occur after enucleation. We therefore developed a technique for isolating a pure population of optic nerve terminal synaptosomes by applying the electron-dense dye Procion brown to the optic nerve. As shown by EM, the dye was subsequently transported to the terminals; these were then separated from unlabeled terminals by differential sucrose-gradient centrifugation. Optic nerve terminal synaptosomes isolated in this way possessed a high affinity uptake system for choline, but not for GABA or glutamate, and also bound α-bungarotoxin. We conclude that ACh is an optic nerve transmitter in the toad, and that ACh, GABA, and glutamate may be transmitters at other non-retinal synapses.

1309 EFFECTS OF SOMATOSTATIN ON FROG SPINAL CORD. Ante L. Padjen. Dept. Pharmacology & Therapeutics, McGill University, Montreal, Quebec H3G 1Y6

Immunohistochemical technique indicates the presence of somatostatin-like reactivity in some primary afferent neurons in mammals (Hokfelt et al., 1976, Neurosci. 1: 131). In the present study effects of somatostatin and analogs were examined in a superfused isolated hemisected frog spinal cord preparation at 15°C. Synaptic and drug evoked root potentials were recorded using sucrose gap technique on spinal roots (cf Barker et al., 1975, J. Physiol. 245: 537). Somatostatin in conc. above 10^{-6}M caused a dose dependent increase in the ventral root (VR) potential obtained by dorsal root (DR) stimulation (DR-VRP) with a several fold increase in amplitude and duration at conc. of 10^{-5}M. This increase appeared after a latency of 15-30 min and outlasted the application by 30-60 min. It did not show any desensitization. At the same time DR potentials were either slightly augmented (DR evoked DRP) or unchanged (VR evoked DRP). Trasylol (0.5 KIU/ml), a proteinase inhibitor, seemed to lower the threshold for somatostatin action. Similar effects were observed with endocrinologically active des-carboxy des-amino analog of somatostatin but not with inactive retro-somatostatin. In the presence of high Mg/low Ca Ringer or tetrodotoxin ($5x10^{-6}$M), somatostatin in the same conc. range produced a small and variable but immediate hyperpolarization (< 1 mV) of VR and DR. This direct action of somatostatin was accompanied by a selective depression of glutamate evoked responses (more than 50% at $5x10^{-6}$M somatostatin), but not of GABA evoked responses, of both VR and DR. The hyperpolarization and depression showed desensitization which was often complete in 20 min. Effects on responses to substance P were variable, presumably because of its prolonged action and the relatively fast desensitization to somatostatin.

These results are consistent with the possibility that somatostatin acts on both pre- and postsynaptic sites in the spinal cord as a neuromodulator.

Supported by Medical Research Council of Canada.

1310 BARBITURATES, ANTI-CONVULSANTS AND γ-AMINOBUTYRATE RECEPTIVE SITES FROM THE MAMMALIAN CENTRAL NERVOUS SYSTEM. E. J. Peck, Jr. and B. R. Lester*, Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Synaptic plasma membranes and junctional complexes derived from bovine cerebral cortex were employed to study the interaction of barbiturate and anticonvulsants with γ-aminobutyrate (GABA) receptive sites. These binding or receptive sites included Na^+-dependent, low affinity ($K_d \sim 10^{-6}$M) sites identified as neuronal transport sites and Na^+-independent, high affinity ($K_d \sim 10^{-7}$M) sites considered to be postsynaptic receptors for GABA. Using ^{3}H-GABA binding and the membrane derived sites above we have previously shown that pentobarbital does not interact directly at either GABA binding site except perhaps at extremely high (10^{-2}M) levels (Brain Res. Bull, 1:595, 1976); however, Ransom and Barker (Brain Res. 114:530, 1976) have demonstrated that pentobarbital potentiated GABA responses in cultured spinal cord neurons. Since these effects might involve pre- or postsynaptic binding or release sites for GABA, we have examined the effects of a wide variety of GABA analogs, barbiturates, convulsants and anti convulsants for their effect on synaptosomal transport and membrane binding of GABA. Pentobarbital, phenobarbital and diphenylhydantoin had no effect on GABA receptive systems. Bicuculline competitively blocks sodium independent (-Na) system while affecting the transport (+Na) site at much higher concentrations. Diaminobutyrate and and nipecotic acid affect only the transport (+Na) site. β-alanine inhibits the -Na system only. The convulsant picrotoxin had no apparent effect on either +Na or -Na systems, however, the convulsant pentylenetetrazole inhibited to 50% both +Na and -Na systems at 100mM. Its physiologically effective range was 10 to 20mM.

From studies such as these of equilibrium binding and synaptosomal transport and release of GABA we hope that the mechanism of action of pharmacologic and therapeutic agents can be explained or a molecular basis at the synaptic level. (Supported by a grant from the NIH, HD08389, and a Research Career Development Award to E. J. Peck, Jr.).

1311 EFFECTS OF BRAIN TRAUMA ON CATECHOLAMINES AND THEIR METABOLITES. E.W. Pelton, II, L.R. Nelson, and R.S. Bourke. Dept. Neurol./ Div. Neurosurg. Neil Hellman Medical Research Building, Alb. Med. Coll. Albany, N.Y. 12208.

Trauma to the central nervous system may cause both immediate damage and delayed hypoxia, edema, and necrosis. These events may involve vasospasm of the microcirculation and result from active or passive release of vasoactive monoamines from "leaky", ischemic, or injured neurons, or from failure of oxygen-requiring metabolism or energy-dependent reuptake. We have investigated effects of brain trauma on levels of catecholamines (CA) and their metabolites; physiologic requirements for changes observed; and the use of a proper reference as edema and hemorrhage affect wet weight and protein. Regional assay of norepinephrine (NE) and dopamine (DA) in experimental animals (rats and cats) was fluorometric. CA metabolites (HVA, DOPAC, and MHPG) were assayed by gas-chromatography using electron capture.

Trauma to cat brains was produced by shaking, resulting in predictable, sustained coma comparable to human disease (Nelson, L. R., Bourke, R.S., J. Neurosurg., submitted). All animals were anesthetized. Test animals were shaken with subsequent controlled hypoxia. Controls received no insult, were made hypoxic only, or were shaken without hypoxia. In some brain regions, NE was significantly diminished following both trauma and hypoxia. DA was diminished in some regions following trauma but not after hypoxia only. HVA and DOPAC levels were decreased in brainstem in hypoxic animals, particularly with trauma, but increased in cerebral hemispheres. The sediment from homogenized brain was assayed for DNA after lipid extraction and dessication by reaction with diaminobenzoic acid dihydrochloride and fluorometry. Using anesthetized rats, following trauma by weight drop to the intact skull (2800 dyne-CM) brains were immediately removed, weighed, sealed in plastic bags, and incubated at 37°C for 15 or 60 minutes. No difference in NE or DA concentrations was noted between test and control animals. However, in rats with brain left in place for 15 or 60 minutes prior to decapitation, NE concentration was diminished by 25% at 60 minutes in traumatized rats only; DA was unchanged.

Our findings suggest that 1) an intact cardiovascular system is necessary for NE to fall following trauma; 2) DNA is a practical, useful and appropriate reference point for expressing levels of catecholamines and their metabolites which obviates problems using wet weight and protein; and 3) hypoxia and/or trauma appear to have effects in some regions of brain on oxygen-requiring metabolism of CAs, or energy-dependent uptake into presynaptic terminals.

(Supported by NIH Grant NS-13042).

1312 AUTORADIOGRAPHIC STUDY OF THE SPECIFIC UPTAKE AND TRANSPORT OF GLYCINE IN IDENTIFIED APLYSIA NEURONS. Christopher H. Price, R.E. Coggeshall, and D.J. McAdoo. Mar. Biomed. Inst. and Depts. of Anatomy and HBC&G, Univ. Tex. Med. Branch, Galveston, TX 7550.

The identified neurons R3-R14 in the parieto-visceral ganglion (PVG) of Aplysia have high endogenous glycine concentrations (Iliffe et al., 1977, J. Neurochem., in press). Previous studies on R3-R14 did not demonstrate measureable quantities of established neurotransmitters; thus, glycine may fulfill this role in R3-R14. We have used standard light and electron microscope autoradiography (AR) techniques to study the uptake and fate of glycine and other amino acids in these cells. After incubation for 1 h in seawater with 8.8 µm ^{3}H-glycine and fixation in glutaraldehyde, R3-R14 contain a mean of 153 developed silver grains/ 100 µm^2 cytoplasmic area. Neighboring neurons contain only 43 grains/100 µm^2. This fourfold difference is eliminated when ^{3}H-glycine incubations were done in Na^+-free seawater or 0.1 mM $HgCl_2$, suggesting that there is an energy-dependent uptake system for glycine operating in R3-R14. Furthermore, glycine uptake is selective because alanine, serine, and leucine were taken up equally by R3-R14 and neighboring cells. R3-R14 differ from the neurosecretory bag cells of the PVG which concentrate significant quantities of all amino acids, including glycine. Radiochromatographic analyses on bag cells indicated that a large proportion of radioactivity taken up was incorporated into protein. In light microscope autoradiograms, silver grains were clustered in discrete areas the cytoplasm of the bag cells in both glutaraldehyde and formaldehyde fixed tissue. Electron microscope AR revealed high grain densities over Golgi complexes, suggesting the packaging of labeled protein into granules. By contrast, grains in R3-R14 from ^{3}H-glycine incubations were evenly distributed. Fixation with formaldehyde greatly reduced grain counts in R3-R14 (59 grains/ 100µm^2). Because formaldehyde fixes free amino acids poorly, it seems likely that most of the R3-R14 glycine is not incorporated into protein. This was confirmed by radiochromatograms of individual R3-R14 cell bodies -- less than 10% of radioactivity was associated with protein. The axons of R3-R14 travel down the branchial nerve. By incubating the PVG for periods up to 7 h and isolating the branchial nerve with a vaseline seal, we found that glycine is transported down the R3-R14 axons in large quantities at a rate of several mm/h. None of the other amino acids were so transported. We are currently determining the rate of transport. The amount of glycine transported is sufficient to allow us to trace the axons of R3-R14 to their terminals. Work supported by USPHS grants NS 12567, NS 11255, and NS 10161.

1313 ONTOGENESIS OF CALCIUM-DEPENDENT GABA RELEASE IN RAT BRAIN. Dianna A. Redburn, Diane S. Broome* and S.J. Enna, Dept. Neurobiol. and Anat., Pharmacology, U. Tex. Med. Sch., Houston, Tx. 77025.

As previously reported (Coyle, J. and Enna, S., Br. Res. 111:119, 1976), the development of the GABA uptake system differs markedly from the linear increase of other presynaptic markers such as GABA levels or glutamic acid decarboxylase activity. The activity of GABA uptake is near adult levels at birth, increases considerably above that of the adult between one and two weeks after birth, then declines toward adult levels. In the present study, the activity of the calcium-dependent GABA release system was found to increase linearly with age from birth to adult. Thus, the increased synaptosomal uptake of GABA observed at 7-8 days after birth is not associated with an increase in the size of the calcium-dependent, potassium-releasable pool. Assaying the amount of GABA released from synaptosomes in the absence of external sodium to inhibit re-uptake of the neurotransmitter, did not alter the results. Electron microscopic analysis of the synaptosomal fractions from 3, 7, 14, 21 day old and adult rats revealed a steady increase in the number of mature synaptosomes present with a concomitant decrease in microsomal contamination. Further analysis of subcellular fractions revealed an even more dramatic peak of GABA uptake activity at 7-8 days in the microsomal fraction than that observed in the synaptosomal fraction. These data suggest that the high level of GABA uptake activity observed in rat brain 7-8 days after birth is not localized to synaptic regions and may serve a function unrelated to synaptic transmission. Supported by USPHS MH-29739, The Huntington Chorea Foundation, PMA Foundation Starter Grant (SJE), and USPHS Grant EYO 1655-02 (DAR).

1314 NEUROTRANSMITTER SYNTHESIS, STORAGE AND RELEASE BY AGGREGATING CULTURES OF RAT CNS. E. Richelson and P. Honegger,* Mayo Fdn., Rochester, MN 55901

Rotation-mediated aggregating culture of mechanically dissociated fetal rat brain (Honegger & Richelson, Brain Res. 109,335-354, 1976) represents a model system for use in neurobiology and psychopharmacology. We have explored the neurochemical potentials of this system and now report further studies with these cultures. For these studies aggregates derived from fetal (15-16d gestation) brain cells and cultured for 4 wks were incubated with certain [^{3}H]precursors; [^{3}H]products were isolated from cells by 2-dimensional thin-layer chromatography. After 1 hr whole brain cultures incubated with [^{3}H]choline (5µM), [^{3}H]L-glutamic acid (GLU)(60µM), or [^{3}H]L-tryptophan (90µM) were found to synthesize per mg protein: 8 pmoles [^{3}H]acetylcholine (ACh), 125 pmoles [^{3}H]γ-aminobutyric (GABA), and 7 pmoles [^{3}H]serotonin (5-HT), respectively. After 4 hrs of incubation with [^{3}H]L-tyrosine (30µM), [^{3}H]dopamine (DA) per mg protein was: 0.7 pmole in whole brain cultures; 0.1 pmole in forebrain (telencephalon); and 8.5 pmole in hindbrain (mesencephalon-diencephalon + rhombencephalon). To determine storage and release of these putative neurotransmitters, whole brain cultures were incubated with [^{3}H]precursors for 4 hrs, washed free of extracellular tritium, and incubated for another 4 hrs in complete growth medium (DMEM, 15% FCS) under the following conditions (Table): no additions (control); veratridine (V) (50µM); tetrodotoxin (TTX) (5µM); V (50µM) + TTX (5µM); or reserpine (R) (4µM).

[^{3}H]PRODUCT	TRITIUM RECOVERED FROM AGGREGATES (% CONTROL)			
ADDITIONS:	V	TTX	V + TTX	R
Acetylcholine	45	125	-	-
GABA	140	80	80	-
Glutamate	60	95	100	-
Serotonin	30	100	-	-
Dopamine	0	220	200	0

The results with veratridine, which selectively increases the permeability of electrically excitable cells to sodium ions, suggest that a large portion of [^{3}H]ACh, [^{3}H]GLU, [^{3}H]5-HT and [^{3}H]DA was stored in a readily releasable form in electrically active cells; while the results with tetrodotoxin, which blocks the action potential, suggest that there was spontaneous electrical activity in cholinergic and dopaminergic cells in these aggregates. The effect of veratridine on GABA was opposite to that on GLU, suggesting that released GLU was utilized to synthesize GABA. Reserpine depletion of [^{3}H]DA suggests that DA was stored by cells in vesicles in these cultures. Finally, these results suggest that this model may be useful for psychopharmacological studies. (Supported by Mayo Fdn. and USPHS Grant DA 1490 and MH 27692).

1315 NET UPTAKE OF L-GLUTAMATE AND GABA BY HIGH AFFINITY SYNAPTOSOMAL TRANSPORT SYSTEMS. Robert Roskoski, Jr. Dept. of Biochemistry, Univ. of Iowa, Iowa City, IA 52242.

Reuptake of neuroactive amino acids by high affinity transport systems in the central nervous system is thought to terminate the neurotransmitter activity of these substances. This notion has been challenged since the homoexchange of synaptosomal and exogenous L-glutamate and the corresponding homoexchange of synaptosomal and exogenous GABA has been demonstrated. We reported that depolarizing media (56 mM KCl, 1 mM $CaCl_2$) lowers the GABA content of synaptosomes. In such synaptosomes, net and apparent (radioactive) GABA uptake are similar (Ryan and Roskoski, J. Pharmacol. Exp. Ther. 200, 285, 1977). We proposed that nerve terminal preparations have a limited storage capacity and when this is approached, influx and efflux occur and homoexchange results. When rat cortical synaptosomes (1 mg protein/ml) are incubated with 10 μM[^{14}C] L-glutamate, net and apparent (radioactive) uptake are similar. When the synaptosome levels are decreased to 0.5 mg protein/ml with a corresponding decrease in capacity, then net uptake becomes a fraction of radioactive uptake (exchange ensues). Furthermore, pre-incubating the synaptosomes with 50 μM L-glutamate increases the synaptosomal content. In these preparations, apparent (radioactive) uptake is less than chemical uptake indicating that exchange is occurring. Net L-glutamate uptake is Na^+-dependent and temperature-dependent. Furthermore, a 1 mM concentration of KCl or RbCl supports net L-glutamate and GABA uptake. LiCl, NH_4Cl, CsCl, and choline chloride are ineffective. L-glutamate and GABA are taken up into osmotically sensitive components. In addition, diaminobutyric acid (but not β-alanine) inhibits net and apparent GABA uptake. This suggests that GABA is taken up into neuronal and not glial elements. The demonstration of net uptake of L-glutamate and GABA by their respective high affinity systems is consonant with the idea that these systems may play a role in neurotransmitter inactivation in the synaptic region (Supported by USPHS Grant NS-11310).

1316 DEVELOPMENT OF CHOLINE ACETYLTRANSFERASE ACTIVITY IN CULTURES OF AUTONOMIC NEURONS--DEPENDENCY ON RAT AGE. C.D. Ross*, M.I. Johnson and R.P. Bunge. Dept. of Anatomy & Neurobiol., Wash. Univ. Med. Sch., St. Louis, Missouri 63110.

Under certain tissue culture conditions adrenergic neurons of rat superior cervical ganglion (SCG) develop several cholinergic characteristics. We here present data indicating that the ability of these cultured neurons to develop choline acetyltransferase (ChAT) activity is dependent upon their age when placed in culture. ChAT activity in SCG explants after 1 mo in culture was highest in explants taken from 2 day old rats (over 60 mmoles/kg. dry wt./hr, 37°) and lowest in explants from adult rats (1 mmole/kg d/hr). The ability of SCG explants to accrue ChAT activity in culture decreased markedly in explants taken from rats greater than 2 days of age; ChAT activities in explants taken from 10, 16 and 42 day old rats were 24, 7, 4 and 2 mmoles/kg d/hr, respectively. Dopa-decarboxylase (DDC) activity in explants after 1 mo in culture did not differ with respect to the age of the rat at explantation. This DDC activity (200-220 mmoles/kg d/hr) in SCG explants *in vitro* is about 70% of the activity in the adult ganglion *in vivo*. All cultures were grown in a medium previously shown to stimulate ChAT activity. The presence of heart muscle, demonstrated to increase ACh synthesis in SCG neurons (Patterson & Chun, 1977) provided an additional 2-fold increase in the specific activities of both ChAT and DDC in SCG explants from neonatal rats; ChAT activity was unchanged in SCG explants from 6 week old rats. These data are strongly supported by morphological studies. With $KMnO_4$ fixation after one month in culture vesicles in axonal varicosities related to explants from 5 day old rats were predominately clear; varicosities in cultures from adult rats contained predominately dense-cored vesicles. These data suggest that a final determination regarding neurotransmitter production is made during a "critical period" relatively late in autonomic neuron development. The SCG neuron expresses its greatest capacity to develop cholinergic mechanisms at about the 2nd postnatal day, coinciding with the time SCG axons begin reaching their target organs. The end of this critical period coincides with the maturation of these adrenergic endings, suggesting that the axonal target may be instrumental in finally determining whether the SCG neurons synthesize norepinephrine or acetylcholine. This period of neurotransmitter plasticity in SCG neurons extends several weeks beyond the time that the last mitotic activity is known to occur in this neuronal population.

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1317 ACTIVATION OF CHOLINE ACETYLTRANSFERASE BY CHLORIDE: A POSSIBLE REGULATORY MECHANISM. Jean Rossier and Philippe Benda*. The Salk Inst., La Jolla, CA 92037 and Collège de France, Paris 5, FRANCE.

An extensive study on the effects of salts on the kinetic parameters of rat brain choline acetyltransferase (ChAc) shows that this enzyme is specifically activated by anions, and that among anions, Cl^- is the more effective one. These results are similar to those obtained for ChAc from the human placenta.

The amplitude of the activation of the rat brain ChAc by salt is particularly striking. Table I shows that the maximum velocity (V_{max}) of the enzyme increases 25 times when 145 mM NaCl was added to the 5 mM tris acetate buffer. In using higher ionic concentration, it was possible to obtain an up to 60-fold increase in V_{max}.

Table I shows also that in the absence of Cl^-, at low ionic strength, acetylcholine (Ach) is a good ChAc inhibitor (K_i = 0.310 mM). The Ach inhibition becomes negligible when Cl^- is increased to 145 mM (Ach K_i = 45 mM). The increase of the Ach K_i is six times more profound than the increase in choline K_m (see Table I).

The Ach concentration of the presynaptic terminals was calculated by Dunant et al. to be as high as 27 mM. Such a high Ach concentration will completely inhibit ChAc at low ionic strength, but in the presence of 145 mM NaCl, ChAc inhibition by Ach will become negligible. It may be concluded that an increase in Cl^- concentration will promote an increase in ChAc apparent velocity by two synergistic mechanisms: ChAc will become less susceptible to Ach inhibition and ChAc V_{max} will increase.

Table I. Kinetic Parameters of Crude ChAc

	Tris acetate 5 mM	Tris acetate 5 mM + NaCl 145 mM
V_{max} (Arbitrary Unit)	1.	25.
K_m acetyl CoA (μM)	0.8	3.5
K_m choline (μM)	22.	540.
K_i acetylcholine (μM)	310.	45,000.

1318 STABILITY OF CATECHOLAMINES IN POST MORTEM RAT BRAIN. Isaac F. Roubein and Larry J. Embree, Veterans Administration Hospital and Department of Neurology, Louisiana State University Medical Center, Shreveport, Louisiana 71130.

Evidence in the literature suggests that catecholamines (CA) in the brain play an important role in the pathogenesis of neuropsychiatric disorders. One approach to the study of these diseases involves the biochemical examination of brain tissues obtained at autopsy, but the main difficulty in such studies is the rapidity of post mortem changes in necropsy material. Reports in the literature indicate that while post mortem changes in the levels of CA in brain tissues have been investigated, the results are frequently conflicting.

The purpose of our study was to determine the post mortem levels of CA in discrete regions of rat brain under conditions simulating those under which human neural tissues become available post mortem. Male Sprague-Dawley rats weighing 200-300 gms were sacrificed by cervical dislocation and left at room temperature (20.5-22°C) for periods of 1 and 2 hours. The brains were left in the intact animals in order to simulate the human post mortem situation. At the end of each period, the rat was decapitated and the brain removed and immediately dissected on an ice cooled glass plate into the following regions: cortex, striatum, pons and medulla, midbrain, and cerebellum.

Spectrofluorometric determinations of norepinephrine (NE) and dopamine (DA) in these regions indicated that the decline in NE levels after 2 hours post mortem, followed this pattern: Pons and medulla > midbrain > cortex > cerebellum. In the same post mortem period striatal DA declined to about two-thirds of that in control animals. Further observations will be reported.

Supported by the Medical Research Service of the Veterans Administration.

1319 KINETIC DATA ON THE INHIBITION OF THE CHOLINE CARRIER BY ACETYLCHOLINE MUSTARD AZIRIDINIUM ION AND ITS CHOLINE ANALOGUE.
B. Jane Rylett* and E. Howard Colhoun* (SPON: Maurice Hirst), Department of Pharmacology, University of Western Ontario, London, Ontario, Canada.

Choline mustard aziridinium ion (ChM Az) produced death in mice and rats causing signs of toxicity similar to those obtained with hemicholinium-3 (HC-3). ChM Az has been shown to inhibit choline transport in erythrocytes (Clement and Colhoun, Can. J. Physiol. Pharmacol. 53, 1089, 1975) and into rat brain synaptosomes (Rylett and Colhoun, Can. Fed. Biol. Soc. 19, 49, 1976; Can. J. Physiol. Pharmacol., in press). The present report deals with the kinetic parameters for the inhibition of choline transport by ChM Az and other Az compounds in rat forebrain synaptosomes. Apparent Michaelis affinity constants and velocities for the sodium-dependent, high-affinity and the sodium-independent, low-affinity choline transport carriers were computed by use of a four parameter non-linear iterative least squares program. This procedure, in conjunction with graphical analysis by the method of Lineweaver and Burk, revealed that ChM Az produced a competitive inhibition of the high-affinity choline carrier, with a Ki of 1×10^{-5}M. The Ki for acetylcholine mustard aziridinium ion (AChM Az), in the presence of eserine sulphate, was about 1×10^{-4}M and showed mixed competitive inhibition. Ethoxycholine mustard aziridinium ion, similar in structure to AChM Az but lacking the ester bond, did not appreciably inhibit choline transport. Evidence was also obtained that ChM Az may produce irreversible inhibition of the high-affinity choline carrier: thus, rat brain synaptosomes incubated in the presence of ChM Az, then washed 1-3 times to remove the inhibitor and resuspended in Krebs-Ringers phosphate buffer showed little reduction of the inhibition of the sodium-dependent, high affinity transport of choline. In contrast, inhibition of choline transport by HC-3 was not persistent, showing reversal when the synaptosomes were washed in a similar manner. The irreversible inhibition of the choline carrier, by ChM Az, in isolated synaptosomes appears to correlate with the results of Clement and Colhoun (Can. J. Physiol. Pharmacol. 53, 264, 1975) who first showed that the HC-3-like effect of ChM Az on the isolated phrenic nerve-rat diaphragm preparation was not abolished by repeated washing. Should ChM Az alkylate the choline carrier then a way may have been found to isolate and study the nature of the transport mechanism from nerve endings.
(Supported by the National Research Council of Canada)

1320 NICOTINE STIMULATION OF THE SEPTAL AREA AND EFFECTS OF α AND β ADRENOLYTIC AGENTS ON SODIUM, POTASSIUM AND URINE EXCRETION.
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The central regulation of Na^+, K^+ and urinary volume excretion in terms of the participation of the septal area is effected through the cholinergic system which, upon stimulation, causes increased Na^+ and K^+ excretion and lower urinary volume excretion (Saad et al., 1975). The α pathway in the cathecholaminergic system may have a facilitating effect on the excretion of the two cations and of urinary volume, and the β pathway, when stimulated, may have the opposite effect (Camargo et al., 1976). It was shown that muscarinic and nicotinic receptors may participate in the cholinergic system, and the use of α and β blockers indicates that natriuresis, kaliuresis and diuresis are partially mediated by the release of cathecholamines in the brain (Saad et al., 1976). The following experimental conditions were used in order to determine which of the two cholinergic pathways may be interacting with the adrenergic system: intraseptal administration of NaCl 0,15 M, and administration of nicotine (100 nmol) alone or preceded by the α blockers dibenamine (100 nmol) and regitin (100 nmol), and the β blocker propranolol (100 nmol).

It was observed that the natriuretic effect of nicotine was significantly reduced by the α blockers, with values returning close to control levels established with saline solution, an effect potentiated by previous injection of propranolol. Similar effects were obtained in respect to K^+ excretion, with regitin producing a clearer blocking effect. As to diuresis, the level determined by nicotine was reduced by the α blockers, with regitin causing a greater reduction than dibenamine, and with propranolol potentiating this effect.

The results as a whole seem to indicate that the cholinergic system interacting with the cathecholaminergic system may act on the regulation of Na^+, K^+ and urine excretion through the nicotinic pathway with α and β receptors.
Supported by Grant FAPESP

1321 MODIFICATION OF THE CAT GANGLIONIC ACTIVITY AND GABA INDUCED DEPOLARIZATION BY PENTOBARBITAL AND FLURAZEPAM.
W. Schlosser*, S. Franco* and A. Kuehn* (SPON: L. Klevans). Res. Div., Hoffmann-La Roche Inc., Nutley, NJ 07110

Conflicting reports have appeared concerning the interaction of benzodiazepines and GABA-ergic pathways (Haefely, et al., ADVANC. BIOCHEM. PSYCHOPHARMACOL. 14: 131-151, 1975; Steiner and Felix, NATURE (London) 260: 346-347, 1976). In contrast there is general agreement that the barbiturates exert their depressant effect, in part, through an enhancement of GABA-mediated inhibition (Ramson and Barker, BRAIN RES. 114: 530-535, 1976; Nicoll, PROC. NAT. ACAD. SCI. 72: 1460-1463, 1975). In experiments using surface electrodes, we observed a depolarization of the cat superior cervical ganglion following intra-arterial injection of pentobarbital (100nM-1μM). This effect was associated with a blockade of synaptic transmission. Bicuculline (150μg) prevented the pentobarbital evoked depolarization; however, synaptic transmission remained at a depressed level. Intra-arterial injection of GABA 15 sec after pentobarbital resulted in a depolarization which summated with that produced by pentobarbital.

Flurazepam (300nM-5μM) consistently produced a depolarization of the cat ganglion and prolonged blockade of ganglionic transmission. Pretreatment with either bicuculline (250μg) or picrotoxin (500μg) reduced the duration of ganglionic blockade. Administration of GABA during flurazepam induced depolarization resulted in an attenuation of the GABA response as compared to the GABA control, while an injection of GABA after the evoked depolarization had the opposite effect, producing an augmentation of the GABA potential.

These data support the suggestion that pentobarbital mimics, to some extent, the action of GABA. In contrast, the effect of flurazepam on ganglia is more complex, consisting of a drug-induced depolarization during which the GABA response is reduced. There then follows a phase of enhancement of GABA-induced depolarization. This latter effect may be associated with either a reduction of glial cell uptake of GABA or a priming action of flurazepam on the postsynaptic membrane.

1322 ELECTRON MICROSCOPE LOCALIZATION OF ^{125}I-α BUNGAROTOXIN BINDING SITES IN THE OUTER PLEXIFORM LAYER OF THE GOLDFISH RETINA.
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^{125}I-α Bungarotoxin (^{125}I-αBTX), a specific ligand for the nicotinic acetylcholine (AcCh) receptor, was prepared from αBTX purified from crude Bungarus multicinctus venom and iodinated with the chloramine-T method. Isolated eyecups from goldfish (Carassius auratus) were maintained in oxygenated Ringer's bicarbonate solutions and incubated with 10^{-9} to 10^{-7} M ^{125}I-αBTX with or without inhibitors (10^{-5} M nicotine or αBTX). The eyecups were then washed, fixed and processed for light and electron microscopic autoradiography with previously published methods.

Light microscopic autoradiography showed that binding of ^{125}I-α BTX was discretely localized to the outer and inner plexiform layers (OPL + IPL) with the highest concentration/unit area in the OPL. This confirms the findings of Yazulla and Schmidt (Vision Res. 76:878-880, 1976). Labeling was suppressed in both layers with nicotine and virtually eliminated with native αBTX. Electron microscopic autoradiograms showed that the OPL label was localized in the mass of horizontal, bipolar and receptor processes that invaginate into rod spherules and cone pedicles. In rod spherules the grains were present when the invaginating processes contained bipolar elements but were absent in sections when only horizontal processes were present. In cone pedicles the grains were somewhat removed from ribbon synapses when triads included only horizontal processes but were adjacent to the ribbon synapses when bipolars were included.

While rod telodendria and horizontal cell processes within rod terminal invaginations and small horizontal processes within cone terminal invaginations cannot be eliminated from consideration as possible receptor sites, we believe the evidence indicates that some bipolar cell processes within the OPL bind ^{125}I-αBTX. If the receptors which bind αBTX in the central nervous system have the same binding properties as those in the neuromuscular junction, then, by analogy, these bipolar processes contain AcCh receptors.

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1323 THE UPTAKE AND ACETYLATION OF DIMETHYLAMINOETHANOL AND ITS EFFECT IN CHOLINERGIC SYSTEMS. E.S. Sears and Alan Sconzert*. Dept. Neurol., UTHSCD, Dallas, TX 75235.

Dimethylaminoethanol (DMAE), an endogenous ethanolamine, is a tertiary analogue of choline which has been proposed as a precursor to choline through an unidentified transmethylation process. Riker has marketed the acetamidobenzoate of DMAE (Deaner^{R}) for many years as a precursor of acetylcholine but clinical trials have given conflicting results. The influence of DMAE on the cholinergic neurotransmitter system was determined in synaptosomal preparations from rodent caudate nucleus and in partially purified choline acetyl transferase (CAT).

These synaptasomes exhibited a dual uptake system for ^{3}H-DMAE which is in range with those reported for ^{3}H-choline. The Na^{+}-dependent high affinity system has an apparent Km of 1.3 x 10^{-7}M, Vmax of 1.03 x 10^{-10}M/mg protein/min, and is inhibited by hemicholinium-15. The low affinity system exhibits an apparent Km and Vmax of 3.21 x 10^{-5}M and 1.25 x 10^{-9}M/mg protein/min, respectively. At concentrations greater than 5 x10^{-6}M, DMAE competitively inhibits choline uptake. DMAE is acetylated by CAT; however, kinetic studies indicate that it has a 5-fold lower reactivity when compared to choline.

DMAE participates in the phospholipid cycle as do other endogenous ethanolamines. The low affinity uptake system which supplies choline for phospholipid synthesis appears to utilize DMAE as well. The high affinity uptake system for choline, proposed as the rate-limiting step in acetylcholine synthesis, acts on DMAE in like fashion. At certain concentrations, DMAE competitively inhibits choline uptake and may thereby alter acetylcholine synthesis. Under conditions of exogenous administration, DMAE may be taken up and acetylated in cholinergic neurons. It remains to be seen if acetylated DMAE could be released as a false neurotransmitter.

1324 DIFFERENTIAL EFFECT OF SUBSTANCE P ON TRIGEMINAL BRAIN STEM UNITS RESPONDING TO TOOTH PULP OR INNOCUOUS OROFACIAL STIMULI. B.J. Sessle, J.W. Hu, G.E. Lucier, and J.L. Henry. Fac. Dent., Univ. of Toronto, and Dept. Res. in Anaesthesia, McGill Univ.,Canada.

Substance P has recently been shown (Henry, Brain Res. 114, 439, 1976) to have a differential excitatory effect on spinal dorsal horn units responding to noxious cutaneous stimuli in the cat. This suggests that substance P may have a facilitatory role to play in spinal nociceptive pathways. The present experiments were undertaken to determine if substance P has a broader role in nociceptive transmission than simply in spinal mechanisms. Its effects were tested on single units recorded in the trigeminal spinal tract nucleus. In cats anaesthetized with chloralose, and paralyzed with pancuronium bromide (Pavulon), single unit extracellular spikes were recorded from the exposed medulla with multi-barrel micropipettes. The central recording barrel was filled with 2.7M NaCl. Peripheral barrels, used for microiontophoretic application, were filled with synthetic substance P (0.8 mM in 165 mM NaCl, acidified to pH 5.5; Peninsula Labs. Inc.), Na-L-glutamate (1M, pH 7.2; Sigma), and control solution (165 mM NaCl, acidified to pH 5.5, used to detect artifacts due to local changes in current and pH at the electrode tip). As previously described (Sessle et al., Can. J. Physiol. Pharmacol. 54, 66, 1976), peripheral bipolar electrical stimuli were delivered to the ipsilateral and contralateral canine tooth pulps and to the exposed ipsilateral infraorbital and superior laryngeal nerves and forepaw; innocuous mechanical, and noxious mechanical and radiant heat stimuli were also applied. All units which were thoroughly tested were excited by glutamate (10-50 nA). Substance P (50-100 nA) also had an excitatory effect, but only on some units. The slow time course of this effect corresponded to that previously reported in other CNS areas. Those units tested to date that were excited by substance P responded to stimulation of the ipsilateral tooth pulp; units responding only to innocuous orofacial stimulation were not excited by substance P. As the tooth pulp is generally considered to be a site from which the only sensation evoked is pain and receives only small-fibre innervation, the differential effects of substance P seen in these experiments is consistent with the earlier suggestion that substance P is involved in chemical transmission in central nociceptive pathways.
(Supported by the Canadian MRC and by the Quebec MRC).

1325 NOREPINEPHRINE AND DOPAMINE IN RAT SEPTUM AFTER MICROWAVE IRRADIATION: EVIDENCE FOR TISSUE DISRUPTION ARTIFACT. Nansie S.Sharpless* and Lucy L. Brown*(SPON:R. Katzman).Depts.Neurology & Psychiatry Albert Einstein College of Medicine, Bronx, New York 10461

Recent reports indicate that post-mortem metabolism of brain catecholamines (CAs)is rapid. Increases in 3-methoxytyramine,the O-methylated metabolite of dopamine (DA) by catechol-O-methyltransferase (COMT),appear within minutes after decapitation (Carlsson et al.,1974). Because rapid heat inactivation of CAdegradatory enzymes such as COMT would allow time for precise dissection of discrete brain regions, we have studied the effect of microwave irradiation (MWR) on levels of DA and norepinephrine (NE) in rat septum and caudate nucleus. Rats were killed by: 1)decapitation at 24 C, 2) 15 sec immersion in liquid N_2, or 3) 6 sec focussed MWR (2kW at 2450 MHz). Brains were removed and placed on an ice-cooled plate under a microscope for dissection. Brain removal and dissection took 10-15 min. Dissections were verified histologically. Tissues were homogenized in ice-cold $HClO_4$, centrifuged, and aliquots of the supernate assayed for NE and DA radioenzymatically. In the septum, mean (±SE) DA levels were markedly and significantly higher ($P<0.001$) in the rats killed by MWR (27.1 ± 2.6 ng/mg protein) than in the rats killed by decapitation at 24C (12.5 ± 1.0) or by liquid N_2 immersion(14.0 ± 1.2). However, when the rats were killed 10 min before MWR, allowing time for post-mortem metabolism, DA in the septum was also increased (34.5 ± 3.9). Also, mean DA levels in the caudate were slightly lower in rats killed by MWR (86.4 ± 5.4) than in rats killed by N_2 immersion (119.2 ± 10.8), but the difference was not significant. In contrast to DA, mean NE levels were significantly lower ($P<0.01$) in the septum of rats killed by MWR (7.2 ± 0.7) than in those killed by decapitation at 24 C (11.7 ± 1.1) or by liquid N_2 immersion (11.4 ± 1.0). The marked increase in DA in the septum after both ante- and delayed post-mortem MWR and the slight decrease in the caudate suggest that DA in the septum had been augmented by influx from adjacent regions of high DA content rather than because of COMT inactivation. Similarly, the decreased NE suggests that NE in the septum had spread out to regions of low concentration. Such diffusion artifacts have also been suggested by Lenox et al. (1976). Further evidence for CA spread was obtained by histofluorescence. After MWR there was considerable cell disruption. The fluorescent CA fibers normally seen in the septum were absent and replaced by a diffuse fluorescence indicating that the membranes had broken and the CAs had spread into the surrounding area. Since 6 sec were needed to completely inactivate COMT in brain, it was not possible to shorten the MWR exposure. We conclude that MWR is not a satisfactory method to prepare brain tissue when regional CA levels are being measured.
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1326 IN SITU U.V. LASER STUDIES OF RAT ARCUATE NUCLEI REVEAL ENDOGENOUS FLUOROCHROMES. David Shoemaker *, Joseph T. Cummins and T. George Bidder* (SPON: Wm. G. Clark.) Addiction Research Lab. and Dept. Psychiatry, V.A. Hospital, Sepulveda, CA., 91343 and Dept. of Pharm. and Expt'l. Ther., Univ. Calif., Irvine, CA.

A recently developed U.V. laser-microfluorimeter has revealed a number of intensely fluorescent endogenous substances with highly discrete neuroanatomical localization in the rat brain. The laser-microfluorimeter utilizes a He-Cd, U.V. laser (325 nm) which excites a brilliant yellow-green fluorochrome in the rat arcuate nucleus which is superfused with Krebs-Ringer. The intense arcuate nucleus fluorescence is observed under a stereomicroscope and measured by a monochromator and photon counting system.

The fluorochrome has an emission peak at 520 nm and fades with continued illumination. Ascorbic acid inhibited this decay, indicating a possible photooxidation reaction. Numerous rat arcuate nuclei were pooled, homogenized and extracted with organic solvents. Thin layer chromatography in three solvent systems of the arcuate extract identified an arcuate fluorochrome as 6-methoxy-1,2,3,4-tetrahydro-β-Carboline (6-MEOTHβC). Identification was further supported by identical emission peaks at 520 nm and concomitant rates of photooxidation between the extract and a 6-MEOTHβC standard.

The effects of in vivo administration of pharmacological agents on the 6-MEOTHβC fluorescence in situ were measured with the photon counting system. Thus, chlorpromazine decreased the fluorescence 70% but reserpine and nialamide did not significantly change the fluorescence. 5,6-dihydroxytryptamine doubled the fluorescence and visual observation indicated a broadening of the area of fluorescence into the periventricular nuclei bordering the third ventricle. Acetaldehyde administration increased the fluorescence 40%. This latter observation is significant because β-carbolines are the condensation products of aldehydes and bioamines.

Both the localization of 6-MEOTHβC in the arcuate nucleus and its response to psychopharmacologic substances indicate a yet undefined neurohumoral function of this compound.

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1327 CHEMICAL TRIGGERING OF DESYNCHRONIZED SLEEP PHENOMENA
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Carbachol, a long-acting cholinomemetic agent, has been found to induce a state resembling desynchronized sleep (D) when injected into the giant cell field of the cat pontine tegmentum (FTG). The drug induced state, called D carb, is characterized by EEG desynchronization, muscular atonia, and rapid eye movements, cardinal defining features of physiological desynchronized sleep. In previous work, interpretation of the potent and enduring drug effects was confounded by long (22 min) and variable latencies to onset of D carb which suggested an initial disruption of the system by the experimental procedure.

Among the possibly disrupting effects of the procedure were physical and chemical changes related to the large volumes (4µL) and complex constitution of the vehicle (artificial csf). In this experiment, we hypothesized that the immediacy and consistency of effects of microinjecting carbachol would be enhanced by decreasing the volume of a more physiologically inert vehicle.

Cannulae were implanted bilaterally in the cat FTG so that their tips lay at HC -6.5, P4.0, and LR 2.0. Carbachol, at a dose of 4µg, was infused at volumes of 1µL and .25µL, and the cat was recorded in a restraint box for 4 hours following the infusion. Carbachol produced D carb for an average of 43% total recording time, which was close to 5 times the value for control runs. The average latency to D carb in carbachol runs was 6.6 min, compared to 41.3 min to D sleep in controls. There was, however, still considerable variability of response in terms of total D carb, length of D carb periods, and latency.

Further improvement was provided by the infusion of .25µL of a 16µg/µL carbachol solution. A total of 3 carbachol infusions were performed, along with 3 controls. The average D carb time was 81%, which was more than 8 times control values. Further, all 3 latencies were under one minute, and all 3 runs were characterized by long periods of D carb (over 2 hours of initial D carb in 2 out of 3 cases).

Our refinements of technique have produced effects of greater magnitude, immediacy, and consistency than previously observed. We feel that this stems from very small volume infusions which chemically stimulate smaller, more homogeneous cell groups, with less functional disruption and less tissue destruction than in our earlier work.

1328 **THE HUMAN PLATELET AS A MODEL FOR THE SEROTONERGIC NEURON: COMPARISON OF KINETIC AND PHARMACOLOGIC PROPERTIES OF SEROTONIN TRANSPORT IN PLATELETS AND NEURONS.**
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The mechanisms whereby human platelets transport serotonin (5HT) were explored by determining the uptake of 5HT at initial velocities over a wide concentration range. Total 5HT transport could be resolved into a saturable high affinity-low capacity active transport system plus nonsaturable passive diffusion. Previous kinetic analyses of 5HT transport into platelets and brain slices have been found to be in error and the correct kinetic constants have been recalculated here. The saturable active uptake of 5HT into human platelets is directly susceptible to inhibition by several pharmacologic agents (ouabain, metabolic inhibitors, tricyclic antidepressants) which do not inhibit the nonsaturable passive diffusion or the nonsaturable granular transport of 5HT. On the other hand, granular binding of 5HT is directly susceptible to inhibition by pharmacologic agents (reserpine, tetrabenazine, N-ethyl-maleimide) which do not directly inhibit saturable active uptake or nonsaturable passive diffusion of 5HT. Quantitative studies of platelet 5HT transport have shown that at low concentrations of 5HT, the pharmacologic and biochemical properties of total 5HT transport are determined mostly by the saturable high affinity active membrane transport system for 5HT; at high concentrations of 5HT, the properties of 5HT accumulation by platelets are determined mostly by the granular storage mechanism. Detailed comparisons of the kinetic, biochemical and pharmacologic characteristics of 5HT transport in platelets and brain support the notion that the platelet can serve as a model for 5HT transport by central nervous system neurons.

1329 ACETYLCHOLINE AND CHOLINE IN THE BLOOD OF NORMAL INDIVIDUALS AND PSYCHIATRIC PATIENTS.
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Recent studies in patients using physostigmine, a cholinesterase inhibitor which increases acetylcholine levels, have furthered the hypothesis that acetylcholine may be involved in the etiology of affective disorders. To ascertain whether an alteration of the central cholinergic nervous system in affective disorders might be reflected in peripheral tissues, analysis of the Ach and Ch contents of blood was carried out. To determine both Ach and Ch, blood was drawn and the enzymes immediately inactivated by injecting a 500 µl aliquot into 1 ml of a formic acid acetone mixture. Butyrylcholine was used as the internal standard and choline was esterified using propionylchloride. Acetylcholine and choline were identified by pyrolysis-gas chromatography and mass spectrometry. Quantitation was accomplished by mass fragmentography by monitoring fragments m/e 58 and m/e 71. Depressed patients (mean age 40.4) range 18-64 met research diagnostic criteria for bipolar depression, unipolar depression, depressive neurosis, or schizophrenia, schizoaffective type with depression, respectively. Control subjects were not depressed, had no history of affective disorder, nor of any psychiatric disorder within the preceding two years, nor any other medical disorder requiring treatment, (mean age 34.7) range 26-60. The value in nmol/ml ± S.D. (number of subjects) of blood for the control volunteers was acetylcholine 0.81 ± 0.45 (33) and choline 9.58 ± 7.11 (33). The values for acetylcholine for all the patients were 2.12 ± 2.14 (24) and choline 10.97 ± 7.8 (23). The acetylcholine in patients differed significantly from the controls at a level of $P<0.005$. When the patients were subdivided, the acetylcholine concentration of the depressive neurosis group was significantly different from the control group, acetylcholine 1.94 ± 1.30 (7) and choline 11.6 ± 9.7 (7). The remaining subgroups, although higher in acetylcholine content than the control, were not significantly different. The values for bipolar were 2.0 ± 2.6 (8), unipolar 2.9 ± 3.2 (5) and schizophrenia 1.8 ± 1.1 (4). For choline, the values were: bipolar 11.5 ± 9.0 (8), unipolar 71.1 ± 9.0 (5), schizophrenia 8.9 ± 1.7 (4) and depressive neurosis 11.6 ± 9.7 (7). At present, we do not know how well the acetylcholine in the blood reflects central cholinergic activity, but the data identifies a significant increase in acetylcholine in the blood over control values in a group of depressed patients.

1330 UPTAKE AND RELEASE OF ^{3}H-NOREPINEPHRINE IN ILEAL MUCOSA.
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Norepinephrine (5 x 10^{-5} M) in rabbit ileum stimulates absorption of Na^+ and Cl^-, decreases the short-circuit current, and within 2 minutes enhances maximum accumulation of cyclic GMP. We have previously found (Gastroenterology 70: 942, 1976) that high concentrations of carbachol (CARB, 10^{-3} M) have a similar effect on mucosal cell transport of ions and cyclic GMP. Our studies were performed with ileal tissue from which the muscle layers and Auerbach's plexus are removed; thereby leaving intact the peripheral autonomic innervation and submucosal plexus. In order to determine how high concentrations of CARB may have a catecholamine-like effect on transport and cGMP, we have examined first whether this preparation has a high affinity uptake for dl-^{3}H-NE (norepinephrine), and second, the release of ^{3}H-NE by CARB 10^{-3} M. The tissues were studied at 37° C in Krebs-Henseleit buffer containing pargyline and in a 95% O_2 - 5% CO_2 environment. ^{3}H-NE in concentrations from 10^{-8} - 10^{-6} M shows uptake which is linear with time up to 20 minutes. The uptake process demonstrates Michaelis-Menten kinetics with an apparent K_m of 3.2 µM and V_{max} of 2.61 nM/gm/hr (n=3). At 10 minutes this uptake process is partially inhibited (% inhibition) by cocaine (30-90 mM): 33-51%, imipramine (30 mM): 48%, and ouabain (1 x 10^{-5} M): 66%; none of these agents completely blocks the ^{3}H-NE uptake (n=5). Release of ^{3}H-NE was studied under similar conditions with tissue preloaded (^{3}H-NE, 5 x 10^{-7} M). KCl (100 mM), known to stimulate release of ^{3}H-NE in other tissues, produces release into the media of 18% (n=6) at 1 minute and 7% at 3 minutes when compared to spontaneous release. CARB 10^{-3} M produces release of ^{3}H-NE of 22% (n=6) at 1 minute, but does not increase release at 2-5 minutes.

The results of these studies indicate: 1) Ileal mucosal preparations demonstrate a high affinity uptake process for ^{3}H-NE which behaves according to Michaelis-Menten characteristics. 2) KCl produces release of ^{3}H-NE from 1-3 minutes, while CARB 10^{-3} M causes release which occurs within the first minute. 3) These data suggest that the effect of high concentrations of CARB on cGMP metabolism and/or intestinal mucosal ion transport may be related to the release of NE.

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1331 CENTRAL AND PERIPHERAL DEFICIENCY OF NOREPINEPHRINE IN PARKINSON'S DISEASE AND THE EFFECTS OF L-DOPA THERAPY. P.F. Teychenne*, C.R. Lake, M.G. Ziegler*, C. Plotkin*, J.H. Wood*, and D.B. Calne*. NINCDS & NIMH, N.I.H., Bethesda, Md. 20014

A central deficiency of dopamine (DA) in parkinsonism has been established. Sensitive and specific techniques of measuring another catecholamine, norepinephrine (NE), a primary neurotransmitter in both the sympathetic nervous system and brain, have been developed. We have measured plasma and CSF NE in 21 patients with idiopathic parkinsonism, 7 who were not on medication and 14 who were taking L-dopa/carbidopa, and also in 25 control subjects of the same age group. Dopamine-beta-hydroxylase (DBH), the enzyme which converts DA (the immediate precursor of NE) to NE, was measured in plasma.

A significant deficit of NE both in the CSF and plasma was found in untreated parkinsonian patients. Supine diastolic blood pressure (BP) was lower in untreated patients compared to controls. All the patients with Parkinson's disease had lower levels of DBH. Treatment with oral L-dopa/carbidopa (1150±14: 115±1 mg/day) raised the concentration of the CSF and plasma NE but did not significantly change plasma DBH activity. Supine diastolic BP was increased significantly, with L-dopa therapy, to normal values.

Our results confirm the central depletion of NE in parkinsonism (ref) and indicate an additional reduction of peripheral NE in Parkinson's disease. These data are consistent with a possible autonomic dysfunction. The reduced central concentration of DA may reflect deficient DA present as an NE precursor, in addition to DA present as a transmitter. We are presently searching for a peripheral DA deficit in parkinsonism. Adequate L-dopa therapy without carbidopa should replenish the NE precursor, DA, and probably increase the central and peripheral NE levels toward normal. Since the patients were also taking the peripheral decarboxylase inhibitor carbidopa, our results suggest that at the doses used peripheral blockade is not complete.

Subjects (N)	Age (yrs)	Plasma NE (pg/ml)	CSF NE (pg/ml)	BP (torr)	Pulse (beats/m)	DBH (units/ml)
Untreated Parks.(7)	57 ±4	189 ±22	137 ±25	115±4 70±2	72 ±4	241 ±69
Treated Parks.(14)	60 ±2	320[1] ±58	362[1] ±145	126±4 81±2[2]	79 ±5	352 ±97
Controls (25)	55 ±1	371[2] ±42	209[1] ±25	121±4 77±2[1]	101 ±5	844[2,3] ±97

1 significantly different from untreated patients; $p<0.5$
2 significantly different from untreated patients; $p<0.001$
3 significantly different from treated patients; $p<0.001$

Hornykiewicz, O. (1966): Pharmac. Rev., 18:925-962

1332 IMMUNOHISTOCHEMICAL LOCALIZATION OF NEUROTENSIN AND ENKEPHALIN IN RAT CNS. George R. Uhl, Michael J. Kuhar and Solomon H. Snyder. Depts. Pharmacology and Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD. 21205

Neurotensin and enkephalin immunoreactivity appear in many areas of the rat brain following localization by immunohistochemical techniques. Using antisera characterized in radioimmunoassay as possessing high affinities and high degrees of specificity for the respective antigens, the indirect immunofluorescence techniques show neuron-like configurations for this immunoreactivity. In unpretreated rats, most specific fluorescence appears in fiber- and terminal-like patterns, while low-intensity fluorescence overlies some cell bodies. Pretreatment of the rats with 50 µg colchicine, given intracerebroventricularly 24-48 hrs before sacrifice, allows improved visualization of peptide-containing perikarya, as Elde and Hökfelt (R. Elde, personal communication) have also shown. Adsorption of sera with the peptides against which they are directed eliminates specific fluorescence.

Areas enriched in enkephalin and neurotensin fluorescence include the substantia gelatinosa zones of the spinal cord and the trigeminal nuclear complex, the nucleus tractus solitarius, the vagal nucleus, the periventricular thalamus, the central amygdala, the median eminence, the medio-basal hypothalamus, the stria terminalis, and the interstitial nucleus of the stria terminalis. Sparse fluorescence corresponding to both peptides is detected in the lateral reticular formation, corpus striatum, and superficial and deep layers of the cerebral cortex. Substantial neurotensin fluorescence also appears in the midbrain tegmentum, just dorsal to the interpeduncular nucleus, and in the lateral aspect of the nucleus accumbens. One of the most striking differences between the distributions of the peptides is found in the globus pallidus, which contains the densest enkephalin fluorescence in the brain, but only sparse neurotensin fluorescence.

In many areas of the brain, such as the basal hypothalamus and the central amygdala, the density of fluorescent cell bodies after colchicine treatment parallels the density of fluorescence seen in unpretreated animals. Though this suggests that many neurons containing these peptides might be interneurons, lesion studies show their involvement in brain pathways. (Supported by USPHS DA-00266 and MH-33128.)

1333 MODULATION OF ACETYLCHOLINE (ACH) RELEASE FROM SYNAPTOSOMES BY OXOTREMORINE (OT) AND OXOTREMORINE METHIODIDE (OTMI). Molly H. Weiler* and Donald J. Jenden. Dept. Pharmacol., Sch. Med., UCLA, Los Angeles, CA 90024.

Muscarinic modulation of ACh release has been observed in the exposed cortex (Mitchell, J.Physiol. 165:98, 1963), cortical slices (Bertels-Meeuws and Polak, Brit. J. Pharmacol. 33:368, 1968; Polak, Brit. J. Pharmacol. 41:600, 1971), and the longitudinal muscle strip of the guinea pig ileum (Kilbinger, N.S. Arch. Pharmacol. 287:47, 1975). In order to examine whether this modulation is exerted on presynaptic muscarinic receptors or through a neuronal feedback loop, the effect of OT on spontaneous ACh release was studied in a crude (P_2) synaptosomal fraction prepared from rat whole brains. The synaptosomal pellet was suspended in 20 vol Krebs phosphate buffer containing 40 µM paraoxon. After a 10 min incubation at 37°C the P_2 suspension was centrifuged, and the supernatant was analyzed by gas chromatography/mass spectrometry (Jenden et al, Anal. Biochem. 55:438, 1973) for ACh and choline released into the medium. Under these experimental conditions the normal rate of ACh released was constant for the time period measured (0.01 nmol ml-1 min^{-1}).

OT (1 µM) significantly reduced the spontaneous release of ACh over a 10 min period by 18%. Maximal inhibition of ACh release occurred with 10 µM OT (22%) since higher concentrations (50 and 100 µM) did not cause a significantly greater inhibition (26% and 24%, respectively). This inhibitory effect of OT (1 to 100 µM) was reversed by the addition of 10 µM atropine to the incubation medium. Atropine (10 µM) alone had no effect on the spontaneous release of ACh, and did not affect the endogenous synaptosomal ACh levels. The addition of 35mM KCl to the incubation medium also blocked the inhibitory effect of OT on ACh release. Although 35mM KCl alone slightly stimulated ACh release (~10%) it also decreased synaptosomal ACh levels by 37%.

A quaternary N-metho salt of OT was also tested for its effect on the spontaneous release of ACh from synaptosomes. OTMI (10 and 100 µM) inhibited ACh release by 29% and 40%, respectively. The inhibitory effect of this compound also was reversed by 10 µM atropine and 35mM KCl.

The data suggest that the phenomenon of muscarinic modulation of ACh release can be studied in a synaptosomal preparation. Because of the nature of this tissue preparation, neuronal feedback inhibition can be excluded as a mechanism of modulation, and the data are supportive of a presynaptic modulation of ACh release via muscarinic receptors. (Supported by USPHS grant MH 17691).

1334 ASPARTIC ACID AND GLUTAMIC ACID DECREASE WHEN PRIMARY AUDITORY TERMINALS DEGENERATE IN THE COCHLEAR NUCLEUS OF THE GUINEA PIG. R. J. Wenthold and R. L. Gulley. Laboratory of Neuro-otolaryngology, NIH, Bethesda, MD 20014.

The putative neurotransmitters, glutamic acid (Glu) and aspartic acid (Asp), decrease in the cochlear nucleus after lesion of the auditory nerve by cochlear ablation. Asp decreases with a time course similar to that of the morphological degeneration of primary auditory terminals. The level of total Asp in the cochlear nucleus has decreased nearly 10% one day after cochlear ablation and more than 30% after two days and remains at this level up to 28 days. Glu, after a slight increase one day after cochlear ablation, decreases about 12% after two days and continues to slowly decrease through 28 days. Other acidic and neutral amino acids measured either are unchanged or increase at two days after cochlear ablation.

Glu and Asp were measured in subdivisions of the cochlear nucleus two days after cochlear ablation. The pattern of decrease was similar for both amino acids. Largest decreases in Glu and Asp were in the posteroventral nucleus, auditory nerve root and the anteroventral nucleus; smaller decreases were in the granule cell region and deep layers of the dorsal cochlear nucleus. The two amino acids showed no decrease in the molecular layer of the dorsal cochlear nucleus.

Glu and Asp were measured in the cochlear nucleus of the waltzing guinea pig, a genetic mutant with progressive degeneration of the cochlea. Morphologically, most spiral ganglion cells and primary auditory terminals degenerate between the ages of 30 and 90 days. Glu and Asp decrease in the cochlear nucleus of the waltzer at this time. While the levels are normal up to 30 days of age, by 90 days, Glu and Asp are 11% and 17% lower, respectively, in the cochlear nucleus of the waltzer compared to the normal. Cochlear ablation causes a 16.7% and 30.3% decrease in Glu and Asp after three days, respectively, in the cochlear nucleus of the 90-day normal guinea pig. However, in the 90-day waltzer, these decreases are only 4.8% and 5.3% for Glu and Asp, respectively. These data suggest that Asp, and possibly Glu, may be concentrated in primary auditory terminals in the cochlear nucleus.

1335 GLUTAMATE DECARBOXYLASE AND GABA-TRANSAMINASE IN HUNTINGTON'S CHOREA. Jang-Yen Wu[1]* and Edward D. Bird[2]* (Spon: D. D. Louie), Dept. Cell Biology, Baylor College of Medicine, Houston, TX 77030[1] and Addenbrooke's Hospital, Cambridge, England[2]

It has been reported from several laboratories that there is a marked decrease in GABA level and glutamate decarboxylase (GAD) activity in certain regions of brain of patients with Huntington's Chorea. Since GAD and GABA-transaminase (GABA-T) have been purified to homogeneity and antibodies specific to GAD and GABA-T also have been obtained in our laboratory, it seems desirable to employ immunochemical methods to establish whether the lower GAD activity in Huntington's tissue is due to a decrease in number of GAD molecules or an inhibition of GAD activity. Frontal cortex and putamen from control tissue and Huntington's tissue were homogenized in double-distilled water containing 1 mM AET, 1 mM EDTA and 0.2 mM pyridoxal phosphate, pH 7.2. The homogenate was centrifuged at 106,000 x g for 45 min and the supernatant thus obtained was used for immunochemical studies. GAD activity in putamen was greatly reduced in Huntington's tissue compared to the normal tissue (2.4 x 10^{-10} vs 6.9 x 10^{-10} moles GABA formed/min/mg protein). While in frontal cortex it was only slightly reduced (4.6 x 10^{-10} vs 5.4 x 10^{-10} moles GABA formed/min/mg protein). GABA-T activity was about the same in Huntington's tissue and normal tissue (frontal cortex, 7.0 x 10^{-9} vs 6.5 x 10^{-9}; putamen 8.6 x 10^{-9} vs 9.4 x 10^{-9} moles glutamate formed/min/mg protein). In immunodiffusion tests, GAD from both Huntington's tissue and normal tissue formed a single precipitin band with antibody against GAD purified from mouse brain and both precipitin bands fused together. The microcomplement fixation curves, in term of the extent of complement fixed and the shape of the curve, were also similar for GAD from both Huntington's tissue and normal tissue. It is, therefore, concluded that the decrease of GAD activity in Huntington's Chorea tissue is probably due to a reduction in number of GAD molecules (loss of GABA neurons) and not due to an inhibition or inactivation of GAD molecules. (Supported in part by grant from Huntington's Chorea Foundation and NIH grant NS-13224).

PLASTICITY

1336 A MONOSYNAPTIC EXCITATORY FIBER SYSTEM STUDIED IN VITRO: EVIDENCE FOR AN ASSOCIATIONAL PATHWAY IN CA1 STRATUM ORIENS(?). Bradley E. Alger*and Timothy J. Teyler. Dept. Pharmacology, Univ. Calif. Sch. Med., San Francisco, Calif. 94143 and Neurobiol. Div., Northeastern Ohio Univ. Col. Med., Rootstown, Ohio 44272.

The in vitro hippocampal slice preparation has been used as a model system for the study of response plasticity in the brain (Schwartzkroin & Wester, Brain Res., 1975, 89, 107-109; Alger & Teyler, Brain Res., 1976, 110, 463-480). In correctly prepared transverse slices of rat hippocampus the principle tri-synaptic circuit described by Andersen et al (Exp. Brain Res., 1971,13, 208-221) is present and functional. The principle circuit is a tri-synaptic path: perforant path fibers to granule cells of the dentate gyrus to (via mossy fibers) hippocampal CA3 pyramidal cells and to (via Schaffer collaterals) hippocampal CA1 pyramidal cells.

This report describes and documents the presence of a major afferent input to the basal dendrites of the CA1 area. Several experiments, including laminar analysis, latency measurements, and tests of frequency following, indicate that a monosynaptic excitatory pathway synapses among the basal dendrites (stratum oriens) of CA1 cells. The results cannot be accounted for by current spreading to adjacent excitatory fiber systems. Microsurgical procedures localize the course of the fibers to the border of stratum oriens and the alveus. Intracellular recordings verify that the system does terminate on the same population of CA1 cells that receive the Schaffer projection. Two recent reports have confirmed some of these findings (Andersen et al, Nature, 1977, 266, 736-737; Lynch et al, Nature, 1977, 266, 737-739).

The major candidate for the identification of this input had seemed to be the hippocampal commissural system. This system is known to originate in the contralateral CA3, to cross in the ventral psalterium and to terminate in the apical and basal layers of CA1 (and elsewhere)(Blackstad, J.Comp.Neurol.,1956,105,417-537; Gottleib & Cowan, J.Comp.Neurol.,1973,149,393-422;Mosko et al, J.Comp.Neurol.,1973,152,163-174; Lynch et al, Nature, 1977,266, 737-739). However, lesions of the ventral psalterium or of the contralateral dorsal hippocampus fail to eliminate the potentials seen in stratum oriens. Other neuroanatomical alternatives are discussed and it is tentatively concluded that the afferents investigated here represent an ipsilateral association system in stratum oriens of CA1. (Supported by NSF grant BMS75-02802).

1337 INFANT RATS: EFFECTS OF CENTRAL GUSTATORY AND RETICULAR FORMATION DESTRUCTION UPON THE ONTOGENY OF SENSORIMOTOR AND REGULATORY BEHAVIORS. C. Robert Almli and David L. Hill*. Dept. Psychol., Ohio Univ., Athens, Ohio 45701.

Central gustatory pathways and reticular formation were bilaterally destroyed in infant male and female albino rats at 10 days of age. From the day of birth (Day-1), through surgery at 10 days of age, and through adulthood, the brain damaged and control rats received a battery of sensorimotor tests each day. The sensorimotor test battery (e.g., orientation to tactile and olfactory stimuli, vibrisseal placing, oral reflex) consisted of 10 tests. Also measured during development were consummatory behaviors following a battery of regulatory tests (e.g., amphetamine and hypertonic saline injections, food deprivation, taste preference).

Following brain damage at 10 days of age, the infant rats stopped suckling and/or feeding and drinking for up to 15 days postlesion, and hand- and tube-feeding of liquid diet was required to sustain life. These rats remained hypodipsic through sacrifice at 150 days of age. Also, body weight of the brain-damaged rats was permanently reduced by 10%. The brain-damaged rats displayed sensorimotor deficits on some tests (e.g., vibrisseal placing) for up to 22 days postlesion, while other tests (e.g., olfactory orientation) revealed little or no dysfunction. All sensorimotor deficits were transitory. These rats also exhibited a variety of deficits (e.g., attenuated water intakes during food deprivation) for the regulatory tests.

The sensorimotor and regulatory dysfunction produced with lateral hypothalamic destruction has been attributed to damage of fiber systems coursing near the LH (e.g., nigrostriatal bundle, trigeminal lemniscus, gustatory and reticular formation pathways). The present results are similar to results reported for catecholamine depletion in infant rats, and although damage to the above systems produces some of the regulatory and sensorimotor deficits seen in infant rats sustaining LH damage, LH destruction produces more severe and persistent dysfunction. Thus, LH destruction hits these fiber systems at a focal point, thereby producing greater deficits than are produced with destruction of individual fiber systems, and/or LH destruction involves other afferent or efferent fiber systems not yet fully specified.

Following central gustatory and reticular formation destruction during infancy, rats display transient sensorimotor dysfunction and permanent regulatory deficits. These results are fairly consistent with results reported for rats sustaining similar brain damage as adults, and therefore the results suggest that these brain systems have attained a degree of functional maturity by the tenth day of life for rats.

1338 HISTOCHEMICAL DEMONSTRATION OF 5-NUCLEOTIDASE IN NORMAL AND DEAFFERENTED RAT CENTRAL NERVOUS SYSTEM. K. D. Barron, G. W. Kreutzberg* and P. Schubert*. Max Planck Institute for Psychiatry, Munich, German Federal Republic.

Adult rats weighing 300-350 g were killed by intracardiac perfusion with 300 ml of cacodylate-buffered (pH 7.4) 4% formaldehyde containing 0.5% sodium chloride. Perfusate temperature was 20-22°C. After 3 hours immersion-fixation at 2-4° blocks of brain were washed 14-16 hrs. in 0.05M cacodylate-0.25M sucrose, pH 7.4, and sectioned at 60 um on a vibratome. For light and electron microscopic examination vibratome sections were incubated 60-90 mins. at 20-22° in a medium containing 1 mM adenosine-5-phosphate, 2 mM $Pb(NO_3)_2$, 5 mM $Mn(NO_3)_2$ and 50 mM tris-maleate, pH 7.0 (8.5% sucrose added for electron microscopy). Additionally 5-nucleotidase activity was mapped in 18 um sections of unfixed brains (10 mins. pre-fixation in calcium-formalin before incubation).

Light microscopically enzyme activity was related to myelinated nerve fibers of neocortex and cingulum and other brain regions. Ultrastructurally reaction product was deposited on axolemma and at the surface of myelin sheaths adjacent to apposed membranes of glial cells and other elements of neuropil.

Additionally lead precipitate resulting from 5-nucleotidase activity occurred in neuropil of olfactory system, caudate nucleus, pallidum, thalamus, hippocampal formation, cerebellar cortex and other sites. Ultrastructurally neuropilar deposits were located intercellularly, especially near boutons and smaller dendrites, often in the vicinity of, but not at or on, synapses. Plasma membranes of astrocytic processes often had associated reaction product, especially around synaptic complexes (e.g. granule cell layer of cerebellum) and small blood vessels. Neuronal perikarya lacked significant enzymatic activity.

Nonspecific deposits (substrate-lacking media) were thinly scattered over the tissue or concentrated over nuclei.

Rats were killed 4, 6, 12, 18, 30 and 52 days after right entorhinal corticectomy. Light microscopically increased enzyme activity appeared at the junction of the inner and middle thirds of the molecular layer of the ipsilateral dentate gyrus at 4 days. By 6 days the outer 2/3 of the molecular layer of the right dentate was conspicuously reactive. Increased staining had subsided at 52 days. Ultrastructurally reaction product in partly deafferented dentate molecular layer occurred intercellularly in the vicinity of boutons and axodendritic synapses and in association with astrocytic processes.

1339 MOVEMENT AFTER EXTENSIVE DORSAL RHIZOTOMY. D. Berman, A. Marti*, B. Koss* & A.J. Berman. Veterans' Administration Hospital,Bronx, N.Y. and Queens College, CUNY, Flushing, N.Y.

A variety of voluntary movements can be performed by monkeys after dorsal rhizotomy of the responding limb, even in the absence of vision. This suggests that purposive movements can be executed without peripheral sensory input, presumably via some form of central feedback mechanism. Before invoking central mechanisms, however, it is necessary to assess the contribution of sources of peripheral afference other than those provided over dorsal roots directly serving the limb. One of these originates in areas of the body contiguous to the deafferented dermatomes, particularly those areas which might be stretched or otherwise stimulated during limb movement.

Six M. Mulatta (Group I) were subjected to intradural section of dorsal roots T1-T12 bilaterally, followed two weeks later by section of dorsal roots C2-C8. Three animals (Group II) were subjected to dorsal rhizotomy C2-T3, our standard deafferentation procedure. All animals were observed for posture, locomotion, climbing and retrieval of small objects from a dexterity board. In addition, a qualitative evaluation of forelimb movements involved in reaching for a target was made in three animals from each group. No differences between groups were noted for any of these behaviors. It was apparent that, for the categories of movement studied, removal of somatic input from areas caudal to deafferentation did not alter the deficit in the forelimbs from that found after C2-T3 deafferentation alone. It was concluded that the maintenance of motor function after dorsal rhizotomy was not dependent on sensory input from these areas.

It remained possible however that information might be available via long tracts, as a result of movements of the hindlegs. An attempt was made, therefore, to section all spinal dorsal roots in one M. speciosa. This was carried out in three stages over a seven month period. C2-T3 roots were sectioned at the initial procedure, T4-T12 at the second and L1 to base of cord at the third. The deficit seen in the forelimbs was similar to and no greater than that found in animals with C2-T3 roots only sectioned. Furthermore it was possible to train this animal to move either of its forelimbs in the absence of vision for food reinforcement. Movements of the two limbs could be alternately trained and extinguished with the expected savings to criterion over successive alternations.

Postmortem examination of animals in groups I and II confirmed completeness of deafferentation. In the 'complete' deafferent, however, several rootlets were inadvertantly spared at the junctional areas between surgical stages, T4 on the right and L7-S1 bilaterally.

1340 ELIMINATION OF SINGLE NEURONS IN THE LEECH C.N.S. BY INTRACELLULAR INJECTION OF PRONASE. Douglas Bowling* and Itzchak Parnas* (SPON: T. Lamb). Dept. Neurobiol. Sch. Med., Stanford University, Stanford, CA 94305

The aim of these experiments is to kill an individual neuron together with all of its processes in the C.N.S. and then to determine whether changes develop in other neurons. For example, will sprouting occur and processes grow to form new synapses, replacing terminals removed by the operation? Even minor changes might be detectable in an invertebrate preparation, such as the leech, where the properties and connections of many identified cells have been described in detail by anatomical and physiological techniques. For such experiments, however, it is not sufficeint simply to remove the cell body of an identified neuron, since in the leech as in other invertebrates there is evidence that the distal processes can survive for weeks or even months. A solution to this is to inject the mixture of proteolytic enzymes, pronase, into the cell body through a micro-pipette. A few hours later the cell loses its resting potential and by 24 hours the peripheral axons fail to conduct action potentials. Eventually the cell body disappears and no recovery of activity can be discerned in its processes for periods of several weeks. As expected, after a specific motor cell has been injected the appropriate reflexes are lost. Anatomical evidence for the spread of pronase is provided by experiments in which the cell is first injected with HRP which is allowed to spread. Subsequently pronase is injected into the same cell. In the resulting picture, little reaction product is seen in the cell body or in processes within the neuropil. However, distant axons in roots and connectives presumably not yet reached by the pronase are black, indicating that the cell had been successfully injected with the HRP. Other cells known to be in close physical and physiological association with the destroyed cell appear to be undamaged anatomically when injected with HRP. The electrical properties and synaptic interactions of these cells also appear to be intact.

Further observations, including electron microscopy, are being made to characterize the direct effect of the pronase on the injected cells and to look for non-specific damage to other cells. Animals that have survived for weeks or months with one or more injected neurons are being examined for signs of change from the normal organization of the C.N.S.

1341 ALTERATIONS IN A SPECIFIC MEMBRANE PHOSPHOPROTEIN FOLLOWING REPETITIVE STIMULATION OF THE HIPPOCAMPUS. Michael Browning,* Thomas Dunwiddie, Willem Gispen[1]* Gary Lynch. Dept. of Psychobiology, U. of Calif., Irvine, Cal. 92717. [1]Institute of Molec. Bio., State Univ. Utrecht, Padualaan 8, Utrecht 2506, The Netherlands.

Hippocampal synaptic pathways demonstrate remarkable synaptic plasticity and there has been considerable interest in the mechanisms which underlie these processes (Nature,266,677,1977). Considerable correlative evidence has been amassed which suggests that phosphoproteins may play a role in mediating synaptic events. We tested the possibility that changes in phosphorylation are involved in modification of synaptic efficacy by using the "in vitro" assay of Routtenberg and Ehrlich (Brain Res. 92,415,1975) to index the state of phosphorylation of synaptic membrane proteins following repetitive stimulation of the Schaffer collaterals in our hippocampal slice preparation. Despite considerable variability between experiments, a single band, in the molecular weight range of 43-45,000 Daltons, demonstrated at least 15% less incorporation of label in stimulated slices when compared to unstimulated controls. Although other explanations are possible, this difference in incorporation of label may reflect a net increase in phosphorylation following repetitive stimulation thus resulting in fewer sites available for incorporation in a subsequent "in vitro" assay. Evidence pertinent to the question of whether this change is functionally tied to post-tetanic potentiation, or heterosynaptic depression (Nature, 266, 737,1977), or, for that matter, to any change in synaptic transmission will be discussed.

1342 COMPETITIVE INTERACTIONS BETWEEN DEVELOPING CILIARY GANGLION CELLS. Larry Burstein*, G. Pilar and L. Landmesser (SPON: W. Chapple). Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268, and Biology Dept., Yale Univ., New Haven, CT 06510.

During embryonic development, half of the avian ciliary ganglion cells normally die. Since this cell death occurs at the time of peripheral synapse formation and is greatly enhanced by prior removal of the target tissues (ciliary muscle, iris and choroidal coat), it has been proposed that the neurons die because they have failed to effectively compete for a limited number of synaptic sites or amount of trophic substances (Landmesser & Pilar, J. Cell Biol. 1976, 68:357). The possibility that cells die because they have not reached the target was ruled out, at least for the ciliary population which innervates the iris and ciliary muscle, by showing that at St.34 all ciliary cell somas can be retrogradely labelled by incubating the iris and ciliary muscle in horseradish peroxidase (HRP). The potential competition between ciliary cells was further studied by taking advantage of the characteristic innervation pattern of the ciliary and iris muscle. The axons innervating the different targets emerge through specific nerves: Branch (Br) I (most lateral), innervates the iris constrictor and 40% of ciliary muscle; Br II (central), 20% of the ciliary muscle and the iris dilator; and Br III (medial), 40% of the ciliary muscle.

Although axons innervating the different targets are selectively grouped into different nerves, retrograde labelling of individual nerves with cobalt or HRP showed this is not associated with the position of the cell somas: these are scattered through the ganglion.

The rationale of these experiments was to remove a portion of the ciliary cells from effective competition to see if the survival of those which remained could be influenced. Intraocular axotomy of all Br at St.32 resulted in rapid death of all parent cells. Section of Br I and II alone resulted in an increased survival of those ganglion cells which sent their axons through the remaining Br III. Retrograde labelling showed the number of cells sending out axons through Br III was 144 $\pm$ 6.4% (+SD) of the contralateral side. In other words, half of the cells that normally die had survived. This was associated with an increase in the peripheral field of Br III tested electrophysiologically.

Therefore, cell death is not an inevitable phenomenon, because cell survival can be influenced by altering the balance of neurons and their interacting targets. Cells also can innervate other areas of the target, not normally available, suggesting competition at the synaptic level. Supported by NIH-NS 10338, NS 10666 and the Univ. of Connecticut Research Foundation.

1343 PROJECTIONS OF THE SUPERIOR CEREBELLAR PEDUNCLE IN RATS AND THE EFFECTS OF NEONATAL HEMICEREBELLECTOMY. Anthony J. Castro. Dept. Anat., Stritch Sch. Med., Loyola Univ., Maywood, ILL. 60153

In the first part of this study, the normal efferent distribution of the superior cerebellar peduncle (SCP) was determined in adult rats using the Fink-Heimer staining technique. Animals sustained stereotaxic transection of the SCP with a small knife and were sacrificed 3 to 9 days postoperatively. Similar to reports on other animals, the SCP (brachium conjunctivum) courses rostrally and decussates in the midbrain to the contralateral side where it bifurcates into ascending and descending limbs. Principal terminations of the contralateral ascending limb are the red nucleus and ventral thalamus. A moderate number of fibers recross in the thalamic commissure and pass to the ipsilateral ventral thalamus. Additional sites of preterminal degeneration are the oculomotor and trochlear nuclei, the nuclei of Darkschewitsch and Cajal, the lateral aspect of the periaquaductal gray extending to the pretectal region and continuing into the superior colliculus, the nucleus of the posterior commissure, the nucleus parafascicularis, zona incerta and the posterior thalamic nucleus. The contralateral descending limb passes caudally to the medulla with projections along the way to the pontine gray, the nucleus reticularis tegmenti pontis, the medullary raphe nuclei and the inferior olivary nuclei.

Ipsilateral descending projections are also observed to emerge from the SCP and to course caudally in a position medial to the spinal trigeminal nucleus. Projections from this bundle pass to the trigeminal motor nucleus and can be traced to the lateral pontine and medullary reticular formation with a few fibers passing medially to the nucleus reticularis gigantocellularis. Additional degeneration is also observed bilaterally within the vestibular nuclei with an ipsilateral bundle passing caudally into the medulla in a position just ventral to the dorsal column nuclei.

In the second phase of this study, SCP projections were examined in adult animals that sustained contralateral hemicerebellectomy at 1 to 3 days of age. In these animals aberrant projections are found to the ipsilateral red nucleus and ventral thalamus. Evidence suggestive of axonal sprouting is also observed within the contralateral oculomotor complex. These animals also display an absence of cells in the contralateral pontine gray and inferior olive and the ipsilateral lateral reticular and lateral cuneate nuclei.

(Supported by NIH Grant NS 13230.)

1344 ENVIRONMENTAL INFLUENCES ON THE FUNCTIONAL ORGANIZATION OF THE SUPERIOR COLLICULUS IN THE GOLDEN HAMSTER. Leo M. Chalupa and Robert W. Rhoades*. Dept. Psych., Univ. Calif., Davis, CA 95616.

The response properties of visual neurons in the superior colliculus were investigated in three groups of hamsters: (1) normally reared animals; (2) visually deprived animals, reared from birth to adulthood in total darkness; and (3) strobe reared animals, raised in an environment illuminated twice every second with a very brief flash of light. For visually responsive cells, the receptive fields were plotted and the following response characteristics were investigated: (a) directional selectivity, (b) speed preferences, (c) response type and maghitude to flashed stationary spots of various sizes, and (d) response habituation.

Dark-reared animals could not be differentiated from normal animals on the basis of the incidence or preferred directions of the directionally selective cells. Furthermore, speed preferences and receptive field size and organization were the same in these two groups. Subtle neurophysiological effects of visual deprivation were indicated by the longer latencies of "on" responses to flashed spots of light in the dark-reared animals. In addition, a few cells in the dark-reared hamsters responded in a sustained fashion to flashed spots of light, while in normal animals all cells responded only phasically to light onset and/or offset.

In contrast, marked changes were apparent in the response properties of collicular neurons of the strobe-reared hamsters. These consisted of a pronounced reduction in the number of cells which showed directional selectivity. There was also a reduction in the number of cells exhibiting response suppression when stimuli larger than the activating region of the receptive field were employed. Further, in the strobe-reared animals many cells in the superficial layers of the colliculus (those dorsal to the stratum griseum intermediale) often exhibited considerable response variability, an observation common for cells in the deeper layers of normal and dark-reared animals, but rare for cells in the superficial layers

These findings indicate that in the golden hamster, visual experience plays a minimal role in the induction or maintenance of the functional organization of the superior colliculus, but an aberrant visual input during development, in this case strobe-illumination, can induce major changes in this system.

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1345 INDUCED HYPEREXCITABILITY IN AN INSECT CENTRAL NEURON. Richard D. Clark* and Melvin J. Cohen (Spon: L. M. Bartoshuk). Dept. of Biology, Yale Univ., New Haven, Conn. 06520.

A mid-saggital cut separating the anterior 1/2 of the metathoracic ganglion in the cricker Acheta domesticus increases the effectiveness of synaptic input onto the dendritic tree of an identified central neuron, the contralateral dorsal longitudinal motor neuron (CDLM). A long thin neurite from the CDLM soma runs across the midline of the ganglion and gives rise to a major dendritic arborization in the contralateral ganglionic neuropile. The axon then leaves the ganglion from the side opposite the cell body. The midline section of the ganglion therefore separates the dendritic arborization and axon (distal segment of the neuron) from the soma and part of the initial neurite (proximal segment). The hyperexcitability of the experimental dendritic tree therefore occurs in the absence of an attached cell body.

The intact CDLM in control animals is not spontaneously active. Extracellular recordings from the axon and intracellular records from the CDLM dendritic arborization indicate that the normal CDLM can only be driven synaptically by two vigorous procedures: 1) grasping and probing of the mouthparts and antennal bases; 2) high voltage electrical stimulation of the rostral nerve cord at 20/sec or greater. Air puffs to the receptors of the head or the anal cerci and single shocks to the rostral cord do not stimulate this neuron in the normal animal.

In contrast, approximately 50% of the experimental animals with mid-saggital ganglionic cuts show bursts of action potentials in response to air puffs to the head or tail region and also to single weak electric shocks to the rostral cord. In some cases spontaneous action potentials could be recorded from the dendritic arborization and axon of the isolated distal segment of the neuron. This evidence of hyperexcitability is present from 5 to 38 days after midline section of the ganglion. Simultaneous extracellular recordings from the identified peripheral axon and intracellular recordings from the dendritic arborization confirmed the identity of the experimental dendritic structure within the neuropile.

Cobalt backfills of the CDLM axon and dendritic tree in experimental preparations indicate that the isolated dendritic arborization may give rise to new neurites in the region where the dendritic arborization was cut. This suggests the possibility that the hyperexcitability of the isolated dendritic tree might be due to synaptic formation on the newly induced dendritic sprouts.

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1346 NORMAL INCREASE OF NEURONAL NUMBERS IN ADULT STINGRAYS. Richard E. Coggeshall, Robert B. Leonard and William D. Willis, Jr. Departments of Anatomy and Physiology & Biophysics and the Marine Biomedical Institute, Univ. Texas Med. Br., Galveston, TX 77550.

The nervous system of the Atlantic stingray, Dasyatis sabina, differs in at least three major respects from the nervous system of mammals. First, the axons of the dorsal and ventral roots do not intermingle in the peripheral nerves. Second, there are essentially no unmyelinated axons in the dorsal roots, ventral roots, and peripheral nerves. Third, and of particular importance for this report, the number of dorsal root axons, ventral root axons, dorsal root ganglion cells and ventral horn, "motor" cells increase steadily as the animal ages. To establish this last point, stingrays of different sizes were compared. In these animals the number of axons and cells mentioned above are proportional to the size of the animal with the number of ventral root axons and ventral horn motor cells increasing two-fold as the animals increase in size from 55 gm to 8.5 kg and the dorsal root axons and dorsal root ganglion cells increasing three-fold in these same rays. This finding would seem to imply that the ray is capable of 1) making new neurons in adult life and 2) wiring them into an already functioning nervous system. It is our hypothesis that the ability of fish to regenerate their central nervous systems is related to their capacity to increase neuronal numbers in adult life. This work was supported by NIH grants NS 11255 and NS 10161, and a grant from the Muscular Dystrophy Association of America.

1347 ULTRASTRUCTURAL PATTERNS IN THE LATERAL POSTERIOR NUCLEUS OF THE GOLDEN HAMSTER. Barbara J. Crain, Department of Anatomy, Duke University Medical Center, Durham, NC 27710.

During normal development, pre- and post-synaptic elements combine to form specific synaptic patterns. In order to examine the relative contributions of the pre- and post-synaptic populations to these patterns, we are studying the ultrastructural organization of the lateral posterior nucleus (LP) and comparing it to the organization that results when a normal input from the superior colliculus is removed and an input from the retina is experimentally induced.

The ultrastructure of LP is characterized by two types of synaptic complexes. The first is a "central dendrite" complex in which numerous pre-synaptic terminals synapse around and along a central dendrite. Nearly all of these terminals are medium-sized, contain round synaptic vesicles, and make multiple short asymmetric synaptic contacts onto the central dendrite. They also synapse onto terminals which contain pleomorphic synaptic vesicles and make short symmetrical or very slightly asymmetrical synapses onto the central dendrite. Terminals with pleomorphic vesicles are also found outside the complex. The second type of synaptic complex, which is sometimes found adjacent to the first, consists of a very large terminal synapsing onto many dendritic appendages of a single large dendrite. These terminals are characterized by their large size, their densely packed round synaptic vesicles, and their numerous mitochondria. They form asymmetric synapses onto appendages as well as non-synaptic filamentous contacts with the dendritic shaft. In addition to the pre-synaptic types just described, a small terminal type is found outside the synaptic complexes. These terminals have many round synaptic vesicles, are usually small, and make long, markedly asymmetric synaptic contacts onto small dendrites.

Sources of some of the axon terminals have been determined. After removal of the superior colliculus, medium-sized terminals inside the "central dendrite" complexes and small terminals outside the complexes undergo electron-dense degeneration. Removal of the posterior neocortex is followed by filamentous degeneration of large terminals and electron-dense degeneration of both small terminals and other as yet unidentified terminals outside the complexes. Rarely, terminals inside the "central dendrite" complexes also degenerate.

Removal of the superior colliculus at birth is followed in a few days by retinal synapse formation in LP, before its characteristic synaptic complexes have appeared. Experiments are in progress to determine the fate of the complexes and to describe the patterns of retinal termination under these experimental conditions. Supported by NINDS Grant #NS-09623 to W. C. Hall.

1348 PLASTICITY AND SPECIFICITY OF CONNECTIONS OF THE CORPUS CALLOSUM IN THE ALBINO RAT FOLLOWING NEONATAL LESIONS. Cassie Cusick* and Ray Lund. Depts. Biological Structure and Neurosurgery, Univ. Washington, Seattle, WA 98195.

The callosal projection of the albino rat connects homotopic regions of cortex in an orderly, point-point fashion. In addition, there is a heterotopic projection from the S1 area to S2. We investigated the properties of the developing callosal "map" to determine whether this system could compress or expand in size disparity experiments similar to those performed in the visual system of fish and amphibia. The corpus callosum first crosses the midline between the 19th and 20th day of gestation, but the axons do not begin their ascent into the cortex until the 3rd postnatal day. Animals from several litters received large lateral cortical ablations within the first 36 hours after birth. Lesions removed various amounts of cortex, usually from the level of the anterior commissure to the occipital region. After 6-18 weeks survival, longitudinal second lesions were placed in the intact hemisphere in positions which should have connected with the ablated cortex. Nonhomotopic, medially displaced degeneration was seen in Fink-Heimer material. The degeneration often is organized into discrete patches in the presumptive layers I-III, suggesting that axons are not simply "dammed up" at the edge of the remaining cortex. Small injections of HRP into the remaining cortex on the ablated side showed that the cells of origin of this aberrant projection are found in the appropriate cortical layers and in all areas of the convexity which normally provide a callosal input. Preliminary experiments on animals with lateral lesions made on fetal day 18 show that the opposite cortex appears to project more heavily onto the remaining fragment than after comparable lesions made at birth. While the callosal pathway shows the capacity to form aberrant connections within a smaller terminal area, its ability to expand appears to be rather limited: small second lesions and small ^{3}H-proline injections into the remaining cortex on the ablated side reveal no heterotopic inter-areal expansion, although the possibility of local spreading cannot be eliminated. In such cases, the projection from S1 to both its homotopic position and to S2 is maintained, indicating that callosal axons will bypass inappropriate deafferented areas rather than innervate them to form an expanded projection. These results demonstrate that size disparity mismatches can occur outside of the retinotectal projection in the developing mammalian central nervous system.

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1349 ANATOMICAL AND PHYSIOLOGICAL PLASTICITY IN SINGLE IDENTIFIED MOTOR NEURONS. P. L. Donaldson* and R. K. Josephson. Dept. Psychobio., U. of Cal., Irvine, CA 92717.

The cricket, Teleogryllus oceanicus, has two excitatory axons to the extensor tibia muscle (ET) in its jumping leg. The two axons are distingushable by the contractions they elicit in the muscle--the fast axon causes large amplitude twitches, the slow axon produces low amplitude, facilitating contractions. The two axons exit the thoracic ganglion by different nerve roots (n.5 for the fast axon, n.3b for the slow) and therefore either of the axons can be sectioned without damage to the other. Sectioning the fast axon denervates a large portion of the middle of the ET but leaves proximal and distal ends of the ET still innervated by the slow axon. 30 to 60 days after sectioning the fast axon, the slow axon field of innervation is significantly expanded presumably indicating sprouting of slow axon terminals to denervated muscle fibers. Animals whose nerves are sectioned when nymphs have more rapid and more extensive sprouting than animals sectioned as adults. Expansion of the slow axon field continues with time until the muscle is reinnervated by a regenerating fast axon, which occurs rarely in animals sectioned as nymphs. Regenerating fast axons replicate the original fast axon field, including fibers normally dually innervated by the fast and slow axon. Interestingly, in 75% of the animals which regenerated a fast axon there were two or more fast axons innervating the ET via n.5 rather than the usual single fast axon. The source of these additional fast axons is yet undetermined.

After sectioning n.5, the tension initiated by stimulation of the slow axon with either single stimuli or bursts of stimuli is greater in the operated leg than in the contralateral control leg. This increase in slow axon tension begins at the time the degenerating fast axon fails to elicit contractions (7-10 days post-operative), which is before there is detectible expansion of the slow axon field, and continues until a fast axon reinnervates the muscle. Following fast axon reinnervation the tension initiated by slow axon simulation falls to the control level even though the slow axon field is still enlarged. A possible explanation for the increased effectiveness of the slow axon is that the fast axon represses slow axon transmission. In support of this hypothesis, we have found that excitatory junctional potentals (ejps) produced by the slow axon do increase when the contractions are larger and return to normal sizes when the contractions initiated by the slow axon return to normal. The fast axon did not have to innervate the same muscle fibers or even entirely regain its normal innervation pattern to exert a repressive influence on slow axon responses.

1350 INNERVATION OF HIPPOCAMPUS BY CO-CULTURED CENTRAL ADRENERGIC NEURONS FROM FETAL MOUSE BRAINSTEM. Cheryl F. Dreyfus, Michael D. Gershon and Stanley M. Crain. Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461 and Dept. of Anatomy, Columbia Univ. Coll. Phys. & Surg., N.Y. 10032.

We have attempted to determine whether central adrenergic neurons in vitro are capable of growing to and establishing functional connections with one of their normal targets, the hippocampus. Explants containing locus coeruleus and explants of hippocampus from 18-day fetal mice were maintained as co-cultures and were also grown separately. After 1-4 weeks these tissues were analyzed by glyoxylic acid-induced histofluorescence for catecholamines, by light and EM radioautography with ^{3}H-norepinephrine (NE), and by electrophysiology.

Brainstem explants continued to exhibit specifically fluorescent adrenergic cell bodies and varicose fibers after 4 weeks in culture. In contrast, no fluorescent cells or neurites could be seen in isolated hippocampal cultures at 2-3 weeks in vitro. However, when the hippocampus was co-cultured with the brainstem for 2 weeks, adrenergic fibers were seen growing out of the brainstem and entering the hippocampus. Co-cultures of brainstem and hippocampus were incubated with ^{3}H-NE (5×10^{-7}M) and the monoamine oxidase inhibitor, nialamide (10^{-5}M). Radioautographic analyses revealed that brainstem neurites which entered the hippocampus took up NE whereas neurites in isolated hippocampal explants did not. EM study of co-cultures showed varicose axon terminals within the hippocampus to be preferentially labeled.

Electrophysiological studies suggested that these adrenergic neurites within the hippocampus were functional. Complex synaptically mediated slow wave discharges could be evoked by electrical stimuli in isolated hippocampal explants. Introduction of the β-adrenergic antagonist, propranolol, at 10^{-7}-10^{-6} g/ml, did not alter these hippocampal discharges. On the other hand, in hippocampal-brainstem co-cultures, these concentrations of propranolol altered the complex hippocampal responses to brainstem or hippocampal stimuli. Similar alterations in hippocampal responses by propranolol also occurred in these co-cultures after acute transection of all brainstem neurites to the hippocampal explant. Therefore, propranolol's action was to inhibit the effect of brainstem fibers at the hippocampal level.

These experiments provide morphological and electrophysiological evidence that adrenergic neurons from fetal mouse brainstem maintained in organotypic tissue culture can grow into and functionally innervate an appropriate central target - the hippocampus.

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1351 CALCIUM INVOLVEMENT IN LONG TERM POTENTIATION IN THE HIPPOCAMPAL SLICE PREPARATION. Thomas V. Dunwiddie and Gary S. Lynch Dept. of Psychobiology, Univ. of Cal.,Irvine, CA 92717

Since calcium has been demonstrated to be involved in a wide range of synaptic processes including transmitter release, frequency facilitation and post-tetanic potentiation in other systems, we sought to determine its involvement in the phenomenon of long term potentiation in the mammalian hippocampus.

Reducing the level of calcium in the bathing media of hippocampal slices from 2.5mM to 1.0mM had several effects on the physiology of this preparation. While the absolute magnitude of synaptically evoked responses (both population spike and EPSP) were diminished only slightly, paired-pulse responses were considerably augmented, particularly at short (less than 25 msec) intervals,and changes were seen in plots of stimulation voltage vs. response amplitude.

During stimulation trains delivered at moderate (e.g. 15Hz) frequencies in 2.5mM media, population spike amplitudes typically showed patterns of facilitation-depression-facilitation. The initial phase of facilitation was paralleled by increases in the dendritic extracellularly recorded EPSP, while the EPSP showed only depression during the following depression-facilitation sequence. Testing during a stimulation train via a heterosynaptic input supports the hypothesis that the initial facilitation is specific to the stimulated synapses, whereas the secondary facilitation is the result of a cell-wide increase in excitability.

Following the cessation of stimulation trains, long term potentiation was observed much more frequently in 2.5mM calcium media (52/60 slices) than in 1.0mM media (13/57 slices), suggesting an involvement of calcium in the development of this effect. As would be expected, increased levels of magnesium were also able to disrupt the potentiation phenomenon still further. Whether this is due to a direct involvement of ionic fluxes of calcium in potentiation per se, or whether it is an indirect consequence of other changes in the physiology of this preparation has yet to be determined.

1352 REGENERATION IN THE RAT SCIATIC NERVE STUDIED BY LABELING THE MOTOR FIBERS WITH AXONALLY TRANSPORTED RADIOACTIVE PROTEINS. David S. Forman and Richard A. Berenberg. Naval Medical Research Institute, NNMC, Bethesda, MD 20014.

Labeling regenerating axons with axonally transported radioactive proteins provides information about the location of the entire range of nerve fibers from the fastest growing ones to those which are trapped in the scar. We have used this technique to study the regeneration of motor axons in the rat sciatic nerve after a crush lesion. After surgically exposing the nerve in the mid-thigh it was crushed twice at the same site for 45 seconds with plastic covered jeweler's forceps. From 2 to 14 days after the crush the lumbar spinal cord was exposed by laminectomy and multiple injections of [^{3}H]proline were made stereotactically in the region of the ventral horn containing the motor neurons which supply the sciatic. Twenty-four hours later the nerves were removed, cut into 2 mm segments, and the distribution of radioactivity along the nerve was measured by liquid scintillation counting. Radioactivity in the regenerating axons distal to the crush typically exhibited a peak of radioactivity due to an accumulation of label in the tips of these fibers. After a delay of 3.2 $\pm$ 0.2 (SE) days, this peak advanced down the nerve at a rate of 3.0 $\pm$ 0.2 (SE) mm/day. The leading edge of this peak, which marks the location of the endings of the most rapidly growing labeled fibers, moved down the nerve at a rate of 4.4 $\pm$ 0.2 mm/day after a delay of 2.1 $\pm$ 0.2 days; this is the same time course as that of the most rapidly regenerating sensory axons in the rat sciatic nerve, measured by the pinch test. Another peak of radioactivity at the crush site, presumed to represent the ends of unregenerated axons or misdirected sprouts, declined rapidly during the first week, and more slowly thereafter. When normalized for differences in the amount of incorporation, the labeling pattern in different nerves on the same postoperative day was similar. Daily injection of triiodothyronine (25 µg/g) did not significantly alter the rate of regeneration measured by this method.

1353 RETENTION OF HABITUATION OF WITHDRAWAL RESPONSES MEDIATED BY THE FUNCTIONALLY TRANSECTED HUMAN SPINAL CORD. Marcus J. Fuhrer. Depts. Rehab. and Psychiat., Baylor Coll. Med., Houston, Tx. 77030.

This study was the first in a series concerned with describing stimulus conditions that facilitate or retard retention of habituation of the lower extremity flexor withdrawal reflex mediated by the transected human spinal cord. Specific purposes were to: 1) establish a retention interval that would permit reliable though not complete retention to be demonstrated; 2) assess alternative quantitative indices of habituation and sensitization for their sensitivity to retention effects; 3) evaluate effects comparatively in terms of spontaneous recovery (assessed by responsiveness to test stimuli preceding renewed repetitive stimulation) and retention (assessed by responding during the series of renewed habituating stimuli); and 4) investigate the degree to which the performance characteristics of individual subjects were consistent in separate experimental sessions. Thirteen persons with relatively long-standing transection of the cervical spinal cord participated in two daily sessions during which identical experimental procedures were conducted. Each individual had been examined neurologically by at least two examiners, and in no case was voluntary motor functioning or sensation detected below the level of the lesion. The stimulus consisted of a 40-msec. pulse train applied intradermally to the plantar surface. Response measures were based upon the integrated EMG activity of the tibialis anterior and rectus femoris muscles. The essential procedure was to stimulate repetitively at a 1/sec. interstimulus interval until EMG responsiveness was extinguished, discontinue habituating stimulation for a 3-min., stimulus-free retention interval, and then reapply the same number of 1/sec. stimuli. A total of five habituating stimulus series were applied, the only variation being that the retention interval between the fourth and fifth series was 42 min.

Reliable retention of habituation was demonstrated, with effects tending to cumulate across the first four series. The degree of initial reflex sensitization observed during repetitive stimulation decreased progressively across the four series. No evidence for retention of habituation was observed in the fifth series which was preceded by the 42-min., stimulus-free interval. There was some evidence that indices of spontaneous recovery were less sensitive to repeated stimulus series than were retention indices. Several of the performance indices showed substantial test-retest stability.

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1354 THE EFFECTS OF SPINAL CORD TRANSECTION IN FETAL AND NEONATAL MICE. John D. Gearhart*, Mary Lou Oster-Granite, and Lloyd Guth. Dept. Anat., U. Md. Sch. Med., Baltimore, Md. 21201

Although regeneration occurs in the CNS of lower vertebrates, many investigators have found such regeneration in the CNS of both neonatal and adult mammals is limited. Since the fetal CNS has a far greater potential for repair and restitution of damaged cell populations, its regenerative capacities may resemble more closely those of lower vertebrates. We have developed reproducible in utero surgical techniques for the exposure and transection of mid- to low-thoracic spinal cords in 12-14 day gestation (vaginal plug = day 0) mouse fetuses of the Swiss and C57BL/6J strains. We have also laminectomized and transected neonatal mice within three days postnatal. Animals transected as fetuses were born by both spontaneous vaginal delivery and caesarian section. The amount and type of motor activity present in the lower limbs of adult animals of both groups have been correlated with the histologic appearance of the spinal cord. Some animals transected as fetuses were autopsied at various gestational and postnatal ages. They were compared histologically with neonatally transected counterparts to determine the pathogenesis and extent of the degenerative process, the nature and extent of scar formation, and the amount of ingrowth of fibers into the transected region. Some operated animals were completely paraplegic; in these mice nerve fibers were not seen crossing the lesion, even though scar tissue formation was minimal. Other mice showed a variable degree of motor function which correlated histologically with a variable number of fibers bridging the operative site. Further studies are presently underway to determine the extent to which the nerve fibers seen crossing the lesion represent fibers left undamaged by the operation or new fiber ingrowth.

1355 RECOVERY AFTER SPINAL NERVE LESIONS: REFLEX RECOVERY AND LOCOMOTION. Michael E. Goldberger, Jocelyn Prendergast and Marion Murray. Medical College of Pa., Phila., Pa. 19129

Reflex and locomotor recovery were examined in cats following unilateral section of spinal nerves L_5, L_6, S_1, S_2,(L_7 spared). The general nature (slowness, weakness) of the initial deficit was the same in all animals; the distribution of that deficit depended upon whether the lumbosacral plexus was prefixed or postfixed. Of the 5 hindlimb tendon reflexes (patellar, medial, and lateral hamstrings, achilles, tibialis anterior, toe jerks) only the knee jerk was always abolished. In prefixed animals, ankle and lateral hamstring jerks were abolished; tibialis and toe jerks were preserved but weak. In postfixed animals, tibialis was abolished but weak, ankle and lateral hamstring jerks remained; toe jerks were variable. Tactile placing was more impaired in the forward direction in postfixed animals (tibialis abolished; backward tactile placing more impaired in the prefixed group (achilles abolished). Impairment of medial and lateral placing showed no correlation with plexus type. Standardized locomotion (for food reward on a 9' runway) was impaired in both groups in that extension of the knee and weight bearing during stance were absent, and all movements were greatly slowed. Postfixed animals all showed slipping backward of the limb during initiation of the step and walked on the dorsum of the toes. Prefixed cats showed collapse of the whole leg during stance but did not walk on the dorsum and the leg did not slip backward. During the 1st week p.o., all spared tendon reflexes became stronger, some became hyperactive and clonic, and locomotion was much faster. The patellar tendon reflex never returned but various thigh muscle responses were elicited by skin and periosteal stimulation, which are not seen normally. The reflexogenous zone for placing spread. The abnormal locomotor pattern of the limb did not change despite the increase in tactile placing. During the second p.o. week, the animals were able, by changing the position of the limb, to use muscles (e.g. abductors) for weight-bearing when muscles usually performing this function (extensors) were paralyzed. At this time, some ankle tendon reflexes which has been abolished returned and then became brisk. This was accompanied by a change in the limb's movement pattern, e.g. when the tibialis reflex returned in a postfixed cat, the leg no longer slipped backward. This implies that the returned reflex determined the step's pattern. Increase of tendon reflex activity appeared to be more important for recovery of locomotion than increased cutaneous reflexes. Reflex and locomotor recovery could, in some instances, be accounted for by peripheral nerve sprouting of the spared L_7 spinal nerve, in others by CNS adaptation. NIH - NS11919 and PVA - P-31-76

1356 ANATOMICAL AND BEHAVIORAL PLASTICITY FOLLOWING PRENATAL REMOVAL OF DORSOLATERAL PREFRONTAL ASSOCIATION CORTEX IN THE FETAL RHESUS MONKEY. Patricia S. Goldman, Thelma W. Galkin*, Lab. of Neuropsych., NIMH, Bethesda, Md. 20014 and Edward Taub, IBR, Silver Spring, Md. 20910.

The presumptive dorsolateral prefrontal cortex was resected bilaterally in a fetal rhesus monkey at E106 (106th embryonic day) with subsequent replacement in utero and caesarean delivery near term (165 days). Cognitive functions dependent upon the integrity of this neocortical region were assessed during postnatal development. The monkey was sacrificed at 2½ years of age and its brain fixed by vascular perfusion and processed for subsequent cytoarchitectonic and hodological analysis.

The prenatally operated monkey grew to 2½ years of age without exhibiting any gross sensorimotor deficits or evidence of impairment on the classical spatial delayed response problems which depend critically upon the integrity of the dorsolateral prefrontal cortex in mature monkeys. Its performance on these tests was within the range of unoperated monkeys rather than severely impaired as in the case of monkeys with the same cortical areas removed as juveniles or adults.

The lesion performed at E106, when brain fissuration has barely begun, were bilaterally symmetrical and similar in size and topography to those performed after birth when the brain has attained the adult fissural pattern. However, the surface of the telencephalon in the monkey operated upon as a fetus exhibited marked changes in gross morphology: anomolous sulci appeared bilaterally on the lateroventral surface of the frontal lobe and some unusual sulci were also observed in locations as remote as the occipital lobe. The cytoarchitectonic composition of the cortex lining these sulci displayed the basic six-layered pattern of primate neocortex.

Perhaps the most significant finding with regard to a possible explanation of functional plasticity is that the parvocellular subdivision of the mediodorsal thalamic nucleus, which normally projects to the dorsolateral prefrontal cortex, shows little evidence of neuron loss, indicating perhaps that the parvocellular neurons have found some other cortical target for projection. This sector of the dorsomedial nucleus always degenerates following comparable prefrontal lesions performed after birth.

Thus, removal of the dorsolateral prefrontal association cortex in a nonhuman primate a full two months before birth can be followed in postnatal life by extraordinary preservation of behavioral function as well as by significant alteration in the gross morphological characteristics and thalamo-cortical connections of the brain.

1357 EFFECTS OF AXOTOMY ON SPIKING AND NON-SPIKING SOMATA OF IDENTIFIED LOCUST NEURONS. C. Goodman* and W. Heitler* (SPON: R. Zucker). Dept. Zoology, UCB, Berkeley, CA 94720

The identified neurons of locusts include (i) 'non-spiking' neurons, (ii) 'spiking' neurons with spiking axons and non-spiking somata, and (iii) 'spiking' neurons with spiking axons and spiking somata. What are the differences in ionic current channels amongst different identified neurons and are these differences stable or plastic throughout the life of the animal? We have initially approached these questions by studying the somata of two 'spiking' neurons: the fast extensor tibiae (FETi) motorneuron with a non-spiking soma and the dorsal unpaired median extensor tibiae (DUMETi) neuron with a spiking soma. We examined the ionic channels of these two neurons in normal adult animals and animals in which the peripheral axons of both neurons were axotomized or treated with colchicine.

We find DUMETi to have a spiking soma with a Na-Ca action potential; the presence of both ions is necessary for generation of an action potential. TEA has little effect on increasing either the amplitude or duration of the action potential. The non-spiking soma of FETi shows delayed rectification. TEA converts the non-spiking soma into a spiking soma with a Na-Ca action potential, very similar to the normal action potential in the soma of DUMETi. Thus, the non-spiking and spiking somata both have sodium and calcium inward current channels and the difference between them may be due to differences in the delayed outward current channels.

Axotomy or treatment with colchicine converts FETi within 4 days into a spiking soma where the major carrier of inward current is sodium ions, although the calcium channels are still present. Evidence supports the notion that increased numbers of sodium channels are added to the soma membrane. Axotomy of DUMETi converts the normal Na-Ca action potential into a predominantly Na action potential. The calcium channels are still present, but increased numbers of sodium channels in the soma membrane result in the sodium ions alone being sufficient to generate an action potential. Evidence is also presented to suggest that a non-spiking region of DUMETi in the neuropil becomes spiking after axotomy, also due to an increased number of sodium channels.

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1358 CHANGES IN PROTEIN METABOLISM FOLLOWING AXONOTOMY: A TWO-DIMENSIONAL ANALYSIS. Michael E. Hall*, David L. Wilson, and George C. Stone. (SPON: Donald McAfee). Dept. of Physiol. and Biophys., Univ. of Miami, Sch. of Med., Miami, Fl. 33152.

The nerve cell response to axonotomy is known to involve changes in cell morphology and protein synthesis. These changes have been viewed by many as reflecting a biochemical reversion to an earlier developmental stage, involving an enhanced synthesis of structural proteins necessary for regeneration, with a concomitant decrease in the synthesis of functional proteins (1). In the present study, changes in protein synthesis during development and following axonotomy were analyzed using a newly developed modification of the two-dimensional gel electrophoretic technique of O'Farrell (2).

The two principal postganglionic nerves emerging from the superior cervical sympathetic ganglia (SCSG) of adult rats were either cut or crushed unilaterally. At intervals ranging from 1 to 117 days after surgery both SCSG were removed, incubated in the presence of ^{14}C-leucine and subjected to two-dimensional protein separation and autoradiography. With this technique, proteins are first separated on the basis of isoelectric point (pH 4 to 8) and then according to molecular weight (MW 10^4 to 10^5). Also, intact SCSG from 1, 2, 7 and 14 day old rats were labeled and analyzed. Changes in the synthesis of specific proteins were determined by optical-density scanning of autoradiographs.

Preliminary analysis suggests that, of the 300 to 400 proteins visualized, only a minority (about 10%) of these proteins exhibit a change in synthesis, relative to the majority of proteins, following axonotomy. Of those that do change, there are many more whose synthesis increases than there are those whose synthesis decreases. Most of those proteins showing an increase after axonotomy exhibited this increase only within the first month after surgery, with synthesis at control levels thereafter. This was true even when reinnervation of target organs did not occur. Comparison of protein synthesis patterns from immature SCSG with those from adult-control or adult-axonotomy SCSG indicated that axonotomy does not represent a reversion to an earlier developmental stage of the ganglion.

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1359 EFFECT OF NEONATAL WHISKER REMOVAL ON THE SmI SOMATOTOPIC MAP OF THE RAT. H.P. Killackey, T.J. Cunningham, and G. Ivy*
Dept. of Psychobiology, Univ. of Calif., Irvine 92717 and Dept. of Anatomy, Medical College of Pa., Phila., Pa. 19129

The rodent's mystacial vibrissae and their cortical representation provide a unique opportunity to examine the developmental interactions between a peripheral receptor and the central structures to which the receptor projects. In the mouse, each vibrissa is related to a discrete group of cells in layer IV of somatosensory cortex. These groups of cells are called "Barrels" and their structural integrity is dependent on the presence of intact vibrissae during development (Van Der Loos & Woolsey,'73). In the rat a similar but less discrete structure exists and electrophysiological evidence suggests a one-to-one relationship between a vibrissa and a "Barrel" (Welker,'71). In addition, both species possess discrete thalamocortical projections whose organization is also dependent on intact vibrissae during development (Killackey, et al.,'76).

In the present study we sought to determine whether the disruptions which follow early vibrissae removal are accompanied by a relative loss of specificity between receptor and central structures. Rats with unilateral vibrissae removal on the day of birth (Row "C" or Rows "B" & "D") were prepared for electrophysiological mapping of unit clusters under urethane anesthesia. On the control side contralateral to the undamaged vibrissae, unit clusters were responsive to stimulation of one vibrissa or of several vibrissae in the same row. In a series of penetrations from caudal to rostral receptive fields progressed from row to row in an orderly fashion (Row "A" to Row "E"). In contrast, on the side contralateral to vibrissae removal, this orderly organization was disrupted. While restricted receptive fields related to the intact vibrissae could still be found, interspersed between these areas were areas which responded to stimulation of several rows of vibrissae. We also found areas between normal receptive fields which did not appear responsive to vibrissae stimulation. These results suggest that the structural abnormalities reported earlier are accompanied by an apparent loss of the strict specificity found in normal rats.

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1360 SPROUTING IMPEDES REGENERATING SYNAPTIC CONNECTIONS. C-P. Ko* and S. Roper. Dept. Anat., Univ. Colo. Med. Cntr., Denver, CO 80262

Nerve regeneration readily occurs in the peripheral nervous system, but in the CNS severed nerve tracts are rarely restored. We have been investigating factors contributing to the lack of successful nerve regeneration using as a model reinnervation of the parasympathetic cardiac ganglion in frogs after partial or total denervation. We found that when one of the two nerves supplying this ganglion was crushed, it took up to one year for the damaged axons to re-establish all their functional connections. On the other hand, when both preganglionic nerves were crushed, reinnervation was rapid. The difference between the two situations is that when only part of the nerve supply was destroyed, remaining intact axons sprouted and innervated the entire ganglion before regeneration occurred. Thus, the presence of remaining normal and sprouted synapses apparently delays the re-establishment of original synaptic connections. These results were obtained by the following experiments.

Neurons of the cardiac ganglion are grouped along right and left branches of the preganglionic vagus nerves traversing the interatrial septum in the frog. When we impaled neurons with microelectrodes in isolated ganglia and recorded responses evoked by stimulating the vagus nerves, we found about 88% of the cells on the left branch were innervated by the left vagus and 52% by the right (via a chiasm between the vagal branches). 7-10 days after crushing the left vagus, the remaining intact right nerve sprouted and innervated every ganglion cell on the left branch.

However, if we waited several weeks after crushing the left vagus to allow it to regenerate, in a proportion of neurons we recorded intracellular responses evoked by stimulating the operated nerve; many of these cells still had functional inputs from the right vagus. For example, from 9-18 weeks after the operation, 19% of the neurons on the left branch were innervated by the regenerating left vagus, and 90% by the right. If we compared these data with those from animals in which both vagus nerves had been crushed, 58% of the neurons were innervated by the left and 72% by the right vagus at comparable times.

We interpret these findings as follows. Regeneration of axons after partial denervation takes place, but these terminals must compete with pre-existing normal and sprouted ones; this somehow delays the re-establishment of a functional synaptic connection. When both nerves are crushed, right and left vagal axons arrive at vacant postsynaptic sites and functional connections are rapidly restored. We are investigating possible mechanisms underlying how the presence of sprouted synapses impedes the restoration of original connections.

1361 SEQUENTIAL REMOVAL OF THE INSULAR-TEMPORAL REGION ABOLISHES PATTERN DISCRIMINATION. B.S. Layton,* A.W. Toga,* S. Horenstein and D.G. Davenport.* Departments of Neurology and Psychology, Saint Louis University, Saint Louis, Missouri 63104.

Sequential bilateral ablation of the insular-temporal region of the cat brain abolished the ability to discriminate temporal patterns of stimuli thus resembling the effect of bilateral simultaneous destruction of this polysensory area. Six adult cats were trained in a double-grill box shock-avoidance situation to detect a change in a continuous auditory temporal pattern from either soft-loud-soft to loud-soft-loud or the reverse. Each tone was 800 hz and 900 msec in duration. The tones were separated by 100 msec of silence and the triads by 2000 msec. The loud tone averaged 88 db/SPL and the soft 68 db/SPL. After achieving criterion performance of 18/20 correct detections on two successive days, the insular-temporal cortex was removed bilaterally in two cats and unilaterally in the other four. In the latter group, two cats were operated on the left side and two on the right. All animals were retrained following a two week recovery period. The bilateral animals failed to regain criterion performance, but the unilateral animals usually did so rapidly. When the latter animals had regained criterion the remaining insular-temporal region was operated and after a fortnight to recover, retraining again instituted. This time the cats failed to achieve criterion after as much as twice the number of trials required for original acquisition. A simple intensity detection task (i.e. soft-soft-soft to loud-loud-loud or the reverse) was quickly mastered. After completion of the training cycle, the animals were deeply anesthetized and killed by formol-saline perfusion. The brains were removed for anatomic verification. Lesions were somewhat smaller than intended and generally invaded some of the underlying white matter.

These results appear to confirm the dependence of temporal pattern perception on the insular-temporal region and suggest that staged lesions with interpolated practice and relatively lengthy recovery times do not attenuate the deficit in temporal pattern discrimination induced by bilateral simultaneous ablation of this region. Additionally, the comparison of these results with the anomalously accelerated rate of learning of this type of sequence discrimination observed by Colavita after bilateral simultaneous lesions of insular-temporal cortex in the three week old kitten suggests that the behavioral plasticity associated with infant lesions need not necessarily occur subsequent to serial lesions in the adult animal.

1362 **REORGANIZATION OF SYNAPTIC CONNECTIONS IN THE RAT SUBMANDIBULAR GANGLION DURING POSTNATAL DEVELOPMENT.** **Jeff W. Lichtman* (SPON: D. Purves). Washington Univ. School of Medicine, St. Louis, Mo. 63110.**

The innervation of neurons in the submandibular ganglion of neonatal and adult rats has been studied with intracellular recording, and light and electron microscopy. Intracellular recordings from neurons in isolated adult ganglia showed that individual ganglion cells are usually innervated by a single preganglionic fiber. In ganglion cells from neonatal animals, however, graded stimulation of the preganglionic nerve elicited multiple steps (about 5 on average) in the excitatory postsynaptic potential. The same result was obtained when the preganglionic fibers were stimulated at their emergence from the brainstem, indicating that neonatal ganglion cells are innervated by several different preganglionic neurons. The number of preganglionic fibers innervating individual ganglion cells gradually decreased during the first few weeks of life, and by about 5 weeks each ganglion cell was generally contacted by a single preganglionic axon. Electron microscopical counts of synaptic profiles per ganglion cell perimeter showed that the number of synaptic contacts made on each ganglion cell actually increased during the period when the number of axons innervating each neuron was decreasing. These results show that in the rat submandibular ganglion there is a reorganization of neuronal connections during the first few weeks of life which results in a transition from multiple to generally single innervation of ganglion cells. The similarity of the reorganization of initial contacts in developing muscle and the immature submandibular ganglion suggests that synapse elimination may be a general feature of neural ontogeny.

1363 CHANGES IN THE PATTERN OF RETINO-TECTAL CONNECTIONS DURING DEVELOPMENT IN THE CLAWED FROG, XENOPUS. Alison Longley* (SPON: A. E. Hendrickson). Dept of Zoology, University of Washington, Seattle, WA 98195.

Connections between the eye and optic tecta of Xenopus laevis were mapped anatomically using Wallerian degeneration following lesion of discrete regions of the eye. Connections were analyzed in adult frogs and tadpoles at stage 51, the stage at which retinal axons have grown into about the rostral one-half of the tectum.

In adults, retino-tectal connections were ordered, with nasal retina mapping to the caudal part of the tectum and temporal retina mapping rostrally. In the tadpoles, within the innervated area of the tectum, the retino-tectal connections were generally organized as in the adults, with the temporal retina mapping to the rostral part of the innervated tectum and nasal retina mapping primarily to the caudal part. But a portion of the nasal fibers consistently projected to the rostral tectum as well. Electron microscopic observations showed degenerating synaptic terminals at both rostral and caudal portions of the innervated tectum after lesion of just the nasal retina. Synaptic degeneration was not seen in control tadpoles. This indicates that initially, the axons from the nasal retina, which must cross over the rostral tectum before reaching their proper contacts at the caudal tectum, make synaptic connections over a very wide area. This suggests that during development, the retinal axons progressively sort themselves out on the tectum, perhaps on the basis of their synaptic contacts, to produce the orderly retino-tectal map seen in adults.

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1364 HETEROSYNAPTIC POTENTIATION OF CA1 NEURONS IN THE HIPPOCAMPAL FORMATION: LONGITUDINAL FIELD POTENTIAL PROFILE ANALYSIS OF NEURONAL POPULATIONS. Walter C. Low* and Spencer L. BeMent, (SPON: E. Kokmen). Bioelectrical Sciences Laboratory, University of Michigan, Ann Arbor, MI 48109

The synaptic plasticity of afferent fibers to neuronal populations of the hippocampal formation has been well documented. Short-term and long-term potentiation of CA1 neurons have been observed by stimulating various afferent systems with a variety of paradigms.

We have used the in vitro hippocampal preparation to determine the activity of CA1 neurons along the longitudinal axis in response to the electrical stimulation of Schaffer collateral fibers. The amplitude of the CA1 "population spike" has been shown to be related to the sum of unitary discharges at a particular location within stratum pyramidale (Andersen, Exp. Brain Res., 13:208-221, 1971). A bell-shaped curve for the longitudinal field potential profile (LFPP) was obtained from plotting the peak-to-peak amplitude of the Schaffer collateral-CA1 (SCH-CA1) "population spike" as a function of the position of the recording electrode along the longitudinal axis. When the initial LFPP was compared with subsequent SCH-CA1 response profiles a decrement in the peak of the profiles was observed which suggests a decrease in the Schaffer collateral activation of the CA1 neuronal population.

Non-tetanic (1 cps) stimulation of the fiber system traversing the fimbria followed by the generation of the SCH-CA1 LFPP resulted in a profile with a peak amplitude similar to that of the initial LFPP. On the other hand, tetanic stimulation of the fimbria (2 seconds at 100 cps) followed by the generation of the SCH-CA1 LFPP resulted in a response profile with a peak 50% greater than that of the initial LFPP. These observations with non-tetanic and tetanic stimulation suggest that a respective reactivation and an enhanced activation of the SCH-CA1 system were induced by the prior stimulation of fibers traversing the fimbria.

CA1 population EPSPs due to Schaffer collateral activation in conjunction with fimbrial stimulation must be analyzed in order to gain a clear interpretation of the synaptic basis of heterosynaptic potentiation.

(Supported by NIGMS Grant GM 01289 and USPHS Grant NS 08470)

1365 ANOMALOUS INNERVATION OF THE HIPPOCAMPAL FORMATION BY PERIPHERAL SYMPATHETIC AXONS FOLLOWING MECHANICAL INJURY. Rebekah Loy and Robert Y. Moore. Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, Ca 92093

In the course of studying the normal central nervous system noradrenergic (NA) innervation of the hippocampal formation (HF) we found evidence of apparent growth of peripheral sympathetic axons into the structure following mechanical lesions of the fornix and anterior hippocampus. 14 days to 3 months following unilateral aspiration transection of the anterior 2-3 mm of the HF, brains prepared by the Vibratome formaldehyde or glyoxylic acid fluorescence histochemical methods exhibit a normal pattern of locus coeruleus (LC) fibers within Ammon's horn and the area dentata. In addition, there is a new plexus of coarse, intensely fluorescent catecholamine axons which have the characteristic appearance of peripheral sympathetic fibers. This plexus extends through all septo-temporal levels of the area dentata and appears to be innervating neural tissue rather than blood vessels. The anomalous fibers are mostly smooth, non-varicose, preterminal axons, but they also form typical varicose structures identical to the terminals of the autonomic ground plexus, particularly in the infragranular hilus, the stratum granulosum and the inner 1/3 of the molecular layer. The anomalous innervation also extends into the CA3 stratum pyramidale and radiatum. All of these regions are extensively deafferented by the fimbria-fornix lesion.

A similar pattern of anomalous innervation is present in rats with bilateral LC lesions, indicating that it does not arise from the normal central NA innervation of the HF. The conclusion that the anomalous innervation arises from peripheral sympathetic axons is confirmed by the effects of superior cervical ganglionectomy in rats with LC ablation. In these brains the HF is nearly devoid of NA innervation and none of the coarse, anomalous innervation remains.

The transection of the anterior HF in this study would certainly traumatize the vasculature and its sympathetic innervation. This, in all likelihood, results in growth of severed peripheral NA-containing axons, which have ready access to the partially deafferented HF. The sympathetic fibers appear as early as 14 days after the transection and persist for as long as 5 months with no significant changes in either density or organization, indicating that this is a stable response to injury. It is not known whether this innervation is functional; if it is, such anomalous growth could have significant implications for the sequelae of injury to the CNS of both experimental animals and man. (Supported by USPHS Grant NS-12080 and postdoctoral fellowship NS-05372.)

1366 NERVE DOMAINS AND SPROUTING IN SALAMANDER SKIN. Lynn Macintyre* and Jack Diamond. M.R.C. Group in Developmental Neurobiology, Dept. of Neurosciences, McMaster Univ. Med. Centre, Hamilton, Ontario, Canada, L8S 4J9.

A quantitative physiological study has been made of the ability of intact cutaneous mechanosensory nerve fields to enlarge into adjacent denervated regions of skin in the salamander hind-limb. In most animals the dorsal limb surface divides into two fields, the anterior innervated by spinal nerves 15 and 16 anterior, and the posterior by nerves 17 and 16 posterior; there is virtually no overlapping of the two fields at the frontier between them. Within the 2 weeks following a partial denervation which leaves only nerve 15 or 17 intact in the limb, a slight increase in the field of the remaining nerve occurs at the frontier zone, and then field enlargement ceases for the following 6 to 7 weeks. Sprouting subsequently resumes, and the foreign territory is progressively invaded. This general character of field enlargement is also exhibited by the other nerves tested (e.g., 16A, 16P, and a sub-branch of 17) but the period of initial enlargement, and the duration of the "static" phase when sprouting ceases, varies for the different nerves. During this static phase however, the intact nerve fibres have not lost their capacity to sprout, and will make up field deficits caused by section of sub-divisions of the parent nerve trunk; they will also regenerate readily after they themselves are sectioned. These and other findings are consistent with the hypothesis that the nerves are allotted domains of body-space (presumably during development), and that their axons are hindered for significant periods of time from responding to the non-specific sprouting stimulus provided by denervated skin outside the domain; there is however no apparent constraint on axonal sprouting in response to similar skin located within the domain of the parent nerve.

1367 ONTOGENETIC TIME-TABLE FOR THE DEVELOPMENT OF THREE FUNCTIONS IN INFANT MACAQUES AND THE EFFECTS OF EARLY HIPPOCAMPAL DAMAGE UPON THEM. Helen Mahut and Stuart Zola*, Psychology Dept., Northeastern Univer., Boston, Mass., 02115.

Adult monkeys with either fornix or hippocampal ablations are impaired on spatial reversal and object discrimination retention tasks. At the same time, their performance is facilitated on object discrimination reversal tasks. We investigated the effects of early brain damage in 8 infants with bilateral fornix sections and 8 with ablations of the hippocampus performed between 42 and 82 days of age. Five unoperated infants, matched for age, and corresponding groups of juveniles served as control animals. Effects of surgery were first assessed post-operatively at the ages of 3 to 6 mos.

Two functions impaired, one unaffected. Early surgery affected performance on two of the three tasks: infants with either fornix or hippocampal ablations were impaired on both the spatial and retention tests. In contrast, the capacity underlying performance on the object discrimination task was unaffected: unlike adult monkeys, infants in either of the two operated groups did not show facilitation of performance.

A comparison between the performance of normal infants on all three tasks with that of normal adult and juvenile monkeys, 4 and 2 yrs of age, respectively, revealed a possible time-table for the development of different functions. Spatial reversal ability is fully developed by 3 to 6 mos., as is the ability involved in retention tests. However, on the object discrimination reversal task, the performance of normal infants, at 3 and even at 16 mos. of age, was significantly worse than that of adult monkeys. The performance of juveniles fitted between that of adult and infant monkeys. It appears, therefore, that the capacity necessary for efficient performance on this task matures fully closer to 3 than to 2 yrs of age.

Thus, the effects of early damage to the hippocampal system correspond to the presence or absence of a mature capacity at the time of surgery. The apparent "sparing" of the capacities involved in object discrimination reversal learning was due to their immaturity at around 2 mos. of age.

Recovery of initially impaired functions was assessed by re-tests at 1 and 2 yrs of age. On the whole, performance of all operated animals improved with time and practice on both the spatial and retention tasks. However, infants appeared to recover at a somewhat slower rate than juveniles with equivalent ablations. Supported by NICHD Grant HD08135.

1368 EFFECT OF PRIOR LESION OF FRONTAL CORTEX ON RECOVERY FROM LATERAL HYPOTHALAMIC DAMAGE AND ON NEOSTRIATAL DOPAMINE. L. Misantone, D. Roundtree* and L. Lombardi*, Anatomy Department, Hahnemann Medical College & Pennsylvania College of Optometry, Philadelphia Pa. 19102.

The loss of body weight and decrease in food intake seen after unilateral damage to the lateral hypothalamus (LH) is due partly to interruption of the dopamine (DA) nigrostriatal projection (NSP, Ungerstedt, '71). Prior interruption of the serotonergic projection of the dorsal raphe nucleus (DRN) to neostriatum does not protect from LH damage, nor do DRN lesions alone lower body weight (Misantone, '76). If made prior to bilateral LH lesion, bilateral FC damage ameliorates weight loss (Glick & Greenstein, '72). Since FC also projects to neostriatum, the sparing from weight loss might be due to sprouting induced within the NSP by the prior cortical damage.

Three days after subtotal unilateral FC ablation, two groups of rats showed equivalent, statistically significant body weight losses, while a third unoperated group's weight did not change. Body weights were equivalent 28-31 days later, when unilateral subtotal LH lesions of the NSP were made in all three groups. Seven days after LH lesion, body weight of a group with ipsilateral LH + FC lesions (263.2 $\pm$ 31.1g.) was significantly greater than that of groups with LH lesion contralateral to FC damage (196. $\pm$ 27.9 g.) or with LH damage alone (220.5 $\pm$ 36.0 g). Food intake followed a similar pattern. The reduction in DA concentration on the LH lesion side was equivalent in the three groups at seven days. Thus, the sparing from weight loss was not due to unequal NSP damage. DA concentrations on the side opposite to the LH lesion in both cortical damage groups was not greater than control. Therefore, increased DA content in response to vacated FC sites did not occur. The protective effect was obtained only with prior FC lesions that were ipsilateral to the LH damage. While lesions of any of the three systems (LH, DRN, FC) would interrupt projections to neostriatum, only LH lesions alone and FC lesions alone significantly reduce body weight. Thus to reduce the effect of LH damage, it appears that the initial lesion must be within a system which overlaps the LH in behavioral function as well as in anatomical projection. (Supported by Sigma Xi and MH 28782-01)

1369 PERSISTENT ENHANCEMENT OF THE MONOSYNAPTIC REFLEX AFTER DORSAL COLUMN HEMISECTION IN THE CAT. F.R. Morales* and E.E. Decima. Dept. Anat., UCLA Sch. Med., Los Angeles, CA 90024.

The aim of this project was to determine whether any changes were present in the synapses of a given axon at various periods after the section of one of its collateral branches. The synapses studied were those between 1A fibers and alpha motoneurons in the cat spinal cord. In the case of the cat, the 1A fibers from the hind leg reach the cord at the L6-S1 level and divide inside the dorsal column (DC) into ascending and descending branches. The primary afferent collaterals involved in the monosynaptic reflex (MR) to be studied arise also from these branches at the same segmental levels (L6-S1). The ascending branches continue inside the DC until segment L3 is reached, level at which they start leaving the DC in order to make synapses with the Clarke's nucleus cells (Lloyd and McIntyre, 1949).

The preliminary surgery consisted in severing the ascending branches of the 1A fibers by performing a DC hemisection at the level of L3 or L4 in adult cats. Final experiments were carried out in decerebrated-low spinal animals, and the MR of the gastrocnemius lateralis and soleus (GLS) motorpools were studied simultaneously in both sides. One month after the DC hemisection, the MR in the operated side was larger than in the contralateral (non-operated) side. This difference increased during periods of post-tetanic potentiation (PTP). Both results were observed up to four months after the operation. However, these effects were not present in animals studied four days after the DC hemisection. Similar effects during PTP had been observed in our pilot experiments with DC hemisection in rats (Decima et al., Neuroscience meetings, 1976). Two groups of control animals were also studied. One group consisted of normal (unoperated) cats and it was used to test the assumption that normal animals have symmetrical MR's (i.e., similar magnitudes of the left and right MR's recorded in the GLS pools). The other control group was a series of cats in which the dorsal root ganglion of the L3 and/or L4 segments had been removed in one side, 30 days or more before the final experiments. The purpose of this group was to assess the possible effect of partial denervation of GLS motoneurons produced by the DC hemisection, since this operation severed not only ascending, but descending branches of primary afferents as well. The results obtained with the two control groups showed similar magnitude in the MR's of the left and right GLS motorpools.

The persistent enhancement of the MR observed after an ipsilateral DC hemisection is tentatively attributed to a change in the synaptic efficacy of the 1A presynaptic terminals produced as a result of cutting 1A fiber collaterals (i.e., the ascending branches). (Supported by NINDS grant #7154.)

1370 A POSSIBLE BASIS FOR SHORT TERM STORAGE. Robert U. Muller* Dept. Physiol., Downstate Medical Center, SUNY, Brooklyn, N.Y. 11203 (Spon. Samuel Feldman)

Thin lipid membranes modified with an antibiotic called monazomycin (Mon) exhibit a strongly voltage dependent conductance which is reminescent of the K+ gating system of nerve membranes. Thus, in response to a step of voltage, the conductance rises in an "S"-shaped fashion to a steady state, while at offset there is an exponential-like decline. Two of the differences between these conductances should be noted. First, the Mon system is much slower; steady states occur after seconds rather than after fractions of a millisecond. Second, the Mon induced conductance does not depend on the presence of membrane bound channels which open or close with voltage. Instead, Mon enters and leaves the membrane as the voltage is altered; a channel is created by the aggregation of several ($\sim$6) individual molecules. The number of channels (and therefore the conductance) depends on the amount of Mon in the membrane. It is this membrane amount which is controlled by voltage. A most interesting facet of the behavior of Mon induced conductance has to do with its ease of entering and leaving the membrane; it appears that the rate constant of movement between membrane and solution is a function of the amount already in the membrane. Thus, at the end of a voltage step the molecules leave rapidly, but, as the membrane concentration declines, further exit becomes more and more difficult. As a result, residual effects of a single stimulus can be seen hours after the conductance has gone to zero, due to trapped individual molecules. Thus, in a nerve membrane, Mon could act as a parallel conductance system with a memory of past voltage changes.

The presence of such a molecule in the cytoplasm of a post-synaptic cell (or released by a pre-synaptic cell along with a transmitter) would allow for synapses whose strength is a function of their history of activity. For instance, if a Mon-like molecule entered the membrane during an EPSP and induced a K+ or Cl^- conductance, the magnitude of subsequent EPSPs would be reduced; thus we would have an habituating synapse. Simply changing the channels to Na^+ selective would produce a potentiating synapse.

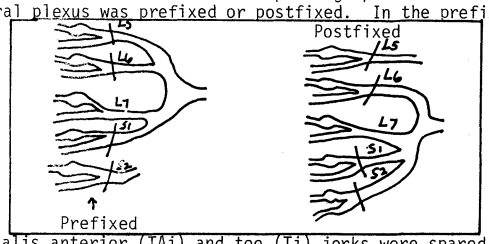
Even more interesting is the possibility of building an excitatory synapse whose strength depends on the closeness in time of an EPSP and a post-synaptic action potential - a "Hebb" or successful synapse. If the synapse were located on an electrically inexcitable dendrite so that the action potential magnitude was attenuated, simultaneous occurrence of an EPSP and an action potential would lead to a much larger voltage change than either one alone, and given the right permeability properties of the newly created channels, to a potentiation of subsequent EPSPs.

1371 REINNERVATION OF PARTIALLY ABLATED TECTUM IN ADULT GOLDFISH. M. Murray and S. C. Sharma. Dept. Anat., Med. Coll. Pennsylvania and Dept. Ophthal., N.Y. Med. Coll., New York, N.Y.

When the caudal half of the tectum is removed from an adult goldfish, the entire retinal projection becomes compressed upon the remaining rostral half tectum. The optic fibers are therefore capable of reorganizing their synaptic connections while retaining their topographical order. In one group of fish, incomplete ablations of the caudal tectum were made so that the caudalmost 1/8 of the tectum was spared in addition to the rostral half. 10-12 months later, they were anaesthetized and evoked potential maps were made of the projection of the contralateral retina upon the partially ablated tectum. The fish were then perfused with a modified Karnovsky fixative and the tecta prepared for electron microscopy. The retina was found to project to both parts of the tectum but the organization of the projection differed. The nasalmost retina projects to the caudal remnant while the remaining retinal projection is compressed upon the rostral half tectum. Together these projections represent the entire retina. The caudal tectal remnant therefore retains the properties which attract the appropriate fibers and this attraction operates over a relatively large distance. The morphology of the stratum opticum and SFGS of the caudal remnant is similar to that of the rostral half tectum. They differ from unoperated tecta in that retinal fibers in the experimental SFGS are organized into fascicles which contain both myelinated and unmyelinated fibers. Thus regeneration into partial tecta is similar to regeneration into the intact tectum. The fibers which innervate the caudal remnant form a distinct bundle which leaves the rostral half tectum, passes caudally over the site of the ablation and enters the caudal remnant. The axons in this bundle at this late post-operative period are mostly myelinated and of large diameter. Axons regenerating into intact tectum are intimately associated with glial cells. In contrast, these axons are embedded in a sheath formed by a loose association of glial and meningeal cells and by abundant fibrillar material. The non-neural cells associated with regeneration of axons to the caudal remnant thus differ from those associated with regeneration into intact tectum. Supported by NS 11644, NEI 01426 and NSF GB43506.

1372 RECOVERY AFTER SPINAL NERVE LESIONS: REFLEX RECOVERY AND SPROUTING. Jocelyn Prendergast, Michael E. Goldberger and Marion Murray. Dept. Anatomy, Med. Coll. Penna., Phila., Pa. 19129.

L_7 spinal nerve was isolated by unilateral section of $L_{5,6}$, and $S_{1,2}$ spinal nerves. On day 1 post-op. two different patterns of tendon reflexes were found depending upon whether the lumbosacral plexus was prefixed or postfixed. In the prefixed class



tibialis anterior (TAj) and toe (Tj) jerks were spared, ankle (Aj) and lateral hamstrings (LHj) jerks were abolished. In the postfixed class the Aj and LHj survived, TA was eliminated. The Tj in the postfixed class was unpredicable. The knee jerk (Kj) was abolished in all cats. Beginning day 3 p.o. patellar tap in both groups elicited abnormal reflex responses including internal rotation and protraction of the hip instead of the normal response leg extention. The new response may be due to an unmasking of an already existing reflex. The residual weakened reflexes became stronger, and some became clonic. Reflexes which were initially absent returned in some cats at ten days. The time course of recovery of lost reflexes was, therefore, greater than that for recovery in strength of spared reflexes. Intramuscular axons and motor end plates were studied by methylene blue staining. At 3 days p.o.: 1) muscles with normal reflex function had equivalent numbers and appearance of axons and end plates on control and denervated sides, 2) muscles with reduced reflexes showed greater numbers of degenerating axons, and 3) muscles with no reflex showed almost total denervation. By the second postoperative week collateral sprouting (increased axon branching) had developed both in muscles with initially weak and initially absent reflexes. The axons of the latter muscles were thinner, often more beaded, fewer in number, and more branched. The more normal appearance of axons in muscles with increased strength suggests that the sprouting is intrinsic (homonymous). Mediation of lost-then-recovered reflexes could be by extrinsic (heteronymous) sprouting and might take longer.

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1373 EFFECTS OF NEONATAL CORTICAL ABLATIONS UPON THE RESPONSE PROPERTIES OF SUPERIOR COLLICULAR NEURONS IN THE GOLDEN HAMSTER. Robert W. Rhoades* and Leo M. Chalupa, Dept. Psych., Univ. Calif. Davis, CA 95616.

We have previously shown (J. Physiol., 1977) that the incidence of directional selectivity for superior collicular neurons in adult hamsters is markedly reduced by acute unilateral removal of the ipsilateral visual cortex. In view of the many recent anatomical demonstrations of reorganization following neonatal damage in the visual system of this, and other rodents, we sought to determine whether or not unilateral removal of posterior neocortex performed during infancy resulted in the same pattern of changes in collicular functional organization as those obtained following similar lesions carried out in adulthood.

All of the hamsters employed in this study underwent complete unilateral aspiration of posterior neocortex on the seventh postnatal day and the responses of visually activated superior collicular neurons were investigated during adulthood (5 to 9 months of age) using conventional extracellular recording and receptive field mapping techniques. In particular, we examined (a) directional selectivity, (b) speed preferences, and (c) response type, magnitude, and latency, to flashed spots of various sizes.

As was the case following lesions performed during adulthood, neonatal removal of visual cortex dramatically reduced the number of directionally selective neurons encountered in the superficial laminae of the ipsilateral superior colliculus. The distribution of preferred directions for those cells which did exhibit selectivity, like that in both normal and acutely lesioned adults, indicated a preference for movement with an upward component. The distribution of speed preferences for the cells tested in the hamsters lesioned during infancy was also different from that observed in normal adults, with the lesioned animals having a greater percentage of cells which yielded responses only to relatively slowly moving stimuli. Responses to stationary, flashed spots for collicular cells in the neonatally damaged hamsters, were, on the other hand, not appreciably different from those which we have observed in normal adult hamsters (Exptl. Neurol., 1977).

These findings suggest that the anatomical reorganization which might be expected to occur following unilateral neocortical ablations in infant hamsters (a question presently under investigation in our laboratory) may not induce corresponding changes in the collicular functional organization of this species.

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1374 LOW FREQUENCY DEPRESSION IN THE IN VITRO FROG SPINAL CORD. Hilliard R. Rogers*, William B. Levy, and Duke E. Cameron*. Dept. of Psychology, UCR, Riverside, CA. 92521 and School of Medicine, Yale University, New Haven, CT. 06520.

The lateral column-ventral root reflex (LC-VRR) of the isolated frog spinal cord displays several forms of homosynaptic plasticity and is currently being studied as a model of behavioral habituation. The experiments presented here attempt to further specify the processes involved in the low frequency depression characteristic of this monosynaptic system.

2M NaCl filled pipettes were used to record extracellular presynaptic and postsynaptic focal potentials from the lumbar motoneuron pool in response to trains of 2,4, or 12 stimuli delivered to the LC at .2 or .5 Hz. Under these conditions the presynaptic potential demonstrated a decrement which closely paralled that of the postsynaptic potential and the VRR. Although presynaptic decrement could be observed in the majority of motoneuron pools sampled, many sites within these same pools did not display the phenomenon. Recordings from the LC during repetitive stimulation showed no decrement in the afferent volley during decrement of the presynaptic potential. In several preparations showing good decrement of presynaptic and postsynaptic potentials synaptic transmission was suppressed by increasing bath levels of magnesium (Mg^{++}). Though the postsynaptic potential was abolished, presynaptic decrement persisted. It was concluded that the observed presynaptic decrement was not due to decrement at the site of LC stimulation, and was independent of calcium (Ca^{++}) coupled transmitter release. In a second series of experiments transmitter release was varied by the use of high Mg^{++}, low Ca^{++}, and high Ca^{++} bathing solutions. Depression of the LC-VRR to trains of 4 or 10 stimuli presented at .2 or .5 Hz was monitored under these conditions. While changes in bath levels of Ca^{++} and Mg^{++} altered LC-VRR amplitude as predicted by the effect of these ions on the transmitter release process, such changes did not alter relative depression to repetitive stimulation.

The results of these experiments suggest that the mechanism of low frequency depression in this monosynaptic system is not one of transmitter depletion, but more likely explained by alterations of processes preceeding the influx of Ca^{++}.

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1375 PLASTICITY IN THE VISUAL SYSTEM OF THE DEVELOPING CHICK. L. A. Rogers*, T. E. Finger and W. M. Cowan. Dept. Anat. and Neurobiol., Sch. Med., Washington Univ., St. Louis, MO 63110.

Chicks in which an unilateral mesencephalic alar plate ablation was performed between Stages 8 and 12 of the Hamburger and Hamilton series (about 36 hours of incubation) exhibit varying degrees of regeneration of the optic tectum. By the time of hatching, the regenerated tectum may have a volume anywhere between 20 and 65% of that found in a normal chick at the same stage of development. In the cases with the most severe loss of tectal tissue, a prominent abnormal retinal projection was observed terminating in the *ipsilateral* area pretectalis, n. lentiformis mesencephali, n. principalis precommissuralis and n. dorsolateralis anterior thalami. To reach these ipsilateral nuclei, the retinal fibers decussate in the optic chiasm and then re-cross the midline in an anomalous pretectal commissure. The magnitude of this aberrant ipsilateral projection appears to be directly proportional to the degree to which the tectum failed to regenerate.

In some chicks, the alar plate was removed unilaterally, together with either the ipsilateral or the contralateral optic vesicle. In these cases the remaining eye has been found to project bilaterally upon both the unregenerated and the regenerated tectum.

These findings resemble rather closely those of Schneider (1973) who has found anomalous projections to the thalamus in hamsters subjected to early partial or complete lesions of the superior colliculus. Both sets of observations are compatible with the view that when deprived of their principal projection field, retinal axons may expand their zones of termination as if to "conserve the quantity of their terminal arborizations". At present we have no evidence that the aberrant retinal projection upon the ipsilateral tectum in our monocular chicks is functional.

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1376 EFFECTS OF PRIOR LONG-TERM TECTAL DENERVATION ON THE RETINAL PROJECTION IN GOLDFISH. Martha Romeskie and S. C. Sharma. Dept. Ophthal., N.Y. Medical College, New York, N.Y. 10029.

In goldfish, immediately following half-tectal ablation or rotation of a piece of tectal tissue combined with optic nerve cut, the regenerating retinal fibers initially project to their original tectal termination sites. Murray (J. Comp. Neur. 168:175, 1976) has proposed that this projection pattern is due to selective attraction between the regenerating axons and the degenerating axonal processes and myelin sheaths of the severed set of optic fibers and their associated glial cells. This debris is present for several months after optic nerve cut. According to this "debris guidance" hypothesis, the initial projection formed by axons regenerating to a tectum lacking degenerating debris should be compressed after half-tectal ablation and normal after tectal rotation. The present study tested these predictions.

The left eye was removed from a group of goldfish. One year later, when no debris is observed in the right tectum, the left tectum was ablated or a piece of the right tectum was rotated. The new projection from the right eye to the ipsilateral tectum was mapped electrophysiologically. In the half-tectum group, all animals in which an ipsilateral projection had formed showed strong evidence of immediate compression; thus the retinal fibers did not appear to return initially to their original tectal sites in the absence of degenerating debris.

However, in the tectal rotation group, the projection to the rotated piece of tissue was correspondingly rotated; the retinal fibers did return to their original tectal sites.

These results suggest that debris guidance may be responsible for the delay in adjustment to a size disparity between retina and tectum, but that it does not affect the retention of the original polarity of the tectum.

Supported by NEI 01426 and NSF GB 43506.

1377 REDUCED RATE OF SYNTHESIS OF DOPAMINE-β-HYDROXYLASE IN NUCLEUS LOCUS COERULEUS DURING THE RETROGRADE REACTION. R.A. Ross, T.H. Joh, and D.J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell University Medical College, New York, NY 10021.

Lesions of the axons of ascending noradrenergic fibers in the posterolateral hypothalamus of rat results in a reversible reduction in the activity and amount of dopamine-β-hydroxylase (DBH)in the parent cell bodies of the nucleus locus coeruleus (LC) 7-21 days following the lesion (Ross et al Brain Res 92:57, 1975). To determine if the reduced accumulation of DBH protein during the retrograde reaction in LC neurons is due to the reduced enzyme synthesis, we followed the rate of incorporation of amino acids into DBH protein in the LC of lesioned and control rats. Electrolytic lesions were placed in the right posterolateral hypothalamus of 10 rats. Twelve days later, during the peak reduction in DBH protein, lesioned rats and unlesioned littermate controls were anesthesized and the IVth ventricle perfused for 30 minutes with 100μl of mock CSF (3.3 μl/min) containing 3H-leucine (s.a. 60 Ci/mmole) or 3H-lysine (s.a. 73 Ci/mmole) and the anesthesia discontinued. At various times thereafter (0,3,and 6 h), the rats were killed and the right LC removed. DBH protein was isolated by immunotitration with a specific antibody to rat DBH followed by SDS electrophoresis. The rate of incorporation of 3H-leucine into both DBH protein and total (TCA-precipitable) protein was linear for up to 6 hours. The rate of incorporation of 3H-leucine into DBH protein in LC of control rats was 358.3±22.6 dpm/h/2 LC. At 12 days following posterolateral hypothalamic lesion, the rate of incorporation was reduced to 221.7±16.4 dpm/h/2 LC, or to 61.8±4.5% of control ($P<.001$). The rate of incorporation of 3H-leucine into total protein did not differ in lesioned and unlesioned rats. Similar results were obtained with 3H-lysine. The percent reduction in 3H-amino acid incorporation into DBH protein closely approximates the percentage decrease in the activity and amount of DBH protein found during the retrograde reaction. We conclude that the reduced accumulation of DBH protein in the LC of rat during the retrograde reaction is due to a decreased rate of de novo synthesis of DBH enzyme protein.

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1378 NEURON-TARGET INTERACTIONS EXPLAIN COMPETITION BETWEEN SALAMANDER MECHANOSENSORY AXONS. Sheryl A. Scott and Jack Diamond. M.R.C. Group in Developmental Neurobiology, Dept. Neurosciences, McMaster Univ. Med. Ctr., Hamilton, Ontario, Canada L8S 4J9.

We know that each rapidly-adapting mechanoreceptor in the dorsal skin of the salamander hind-limb is functionally innervated by only one parent axon, and is morphologically comprised of a single Merkel cell with a few closely apposed nerve terminals; each cutaneous mechanosensory axon supplies a number of such "touch spots". We have now investigated competition between axons for individual sensitive spots: (i) a "foreign" and an appropriate nerve were sectioned and encouraged to regenerate simultaneously into a denervated region of skin, and (ii) a cut nerve was permitted to regenerate into its own skin, which at the same time was becoming innervated by collateral sprouts of neighbouring intact axons. Mechanosensitive spots subsequently reappeared with a density and threshold range similar to that in normal skin, and as usual each spot was innervated by only one axon. Regenerating axons show no obvious preference for their original skin region, and indeed can be excluded from it by collateral innervation from neighbouring intact fibres. Those axons which arrive first appear to have the advantage, and establish their exclusive territory down to the level of the individual touch spots. We have provided evidence that in the salamander skin the targets for mechanosensory nerve endings are Merkel cells, and that these cells survive denervation (Cooper, Scott, Diamond: Soc. for Neurosciences Symp., 1976). Our new results support the indications that Merkel cells exert an attractive influence on cutaneous mechanosensory nerves in the salamander, and that this influence disappears when the Merkel cell becomes innervated.

1379 AN ELECTRONMICROSCOPIC STUDY OF THE MATURATION OF REINNERVATING SYNAPSES WITHIN THE SPINAL CORD OF THE ADULT CAT. R. H. Seall*, and R. E. Kingsley. South Bend Center for Med. Ed., Indiana University, South Bend, IN. 46556.

Ventral roots of L_7 were grafted to the ganglionectomized dorsal roots of L_6 in the spinal cord of adult cats according to the method of Barnes and Worrall. The underlying ultrastructural characteristics of this phenomenon during the course of reinnervation have been previously described (Seall & Kingsley). The present study characterizes synapse maturation in vivo as compared with synapse formation in tissue culture (Rees et. al.).

Two types of new synapses, those resulting from reinnervation of the spinal cord by the ventral roots, and those due to presumed axonal sprouting have been observed. They were differentiated by section of the graft prior to fixation, and noting the resultant signs of degeneration in the boutons derived from reinnervating axons. These two types of immature synapses exhibit the same pattern of maturation.

The most immature synapses which we have observed have a synaptic cleft of 150A, few vesicles, and some post-synaptic specialization. This corresponds to a stage in tissue culture which is approximately 24 hours following the initial contact between the growth cone and the neuron. We have observed both round(350A) and oval (180Ax550A) vesicles within a single immature bouton, along with occasional large (700A) dense core vesicles. As the synapse matures, the synaptic cleft widens to between 220A and 280A, a pre-synaptic density develops, and the large dense core vesicles disappear. Also, during the maturation process, the number of vesicles within the bouton increases and they assume a homogenious shape. While we have observed mature boutons containing only round or only oval vesicles, those derived from reinnervating axons all appear to have only round vesicles. In general, the maturation of developing synapses in vivo, whether derived from reinnervating axons, or from axonal sprouts, is similar to that which has been reported in tissue culture.

1) C. D. Barnes & N. Worrall; J. Neurophysl. 31:689 (1968)
2) R. H. Seall & R. E. Kingsley; Submitted to Proc. EMSA (1977)
3) R. P. Rees, et. al.; J. Cell Biol.68:240- (1976)

1380 LESION-INDUCED PLASTICITY OF HIGH AFFINITY CHOLINE TRANSPORT IN THE DEVELOPING RAT FASCIA DENTATA. David L. Shelton*, J. Victor Nadler and Carl W. Cotman, Dept. Psychobiol., Univ, Calif., Irvine, CA 92717.

After removal of the perforant path input to the rat fascia dentata at the age of 11 days, cholinergic septohippocampal fibers invade the denervated area. We have now examined the effect of this lesion on high affinity choline transport, a specific property of presynaptic cholinergic membrane. For this purpose, particulate fractions prepared from molecular (denervated) and granular layers of fascia dentata were incubated at 38°C with 0.1 μM [^{3}H] choline in the presence or absence of 0.8 μM hemicholinium (a selective inhibitor of high affinity transport at this concentration). Equivalent layers from the fascia dentata contralateral to the lesion served as controls.

Unilateral removal of the perforant path fibers increased the initial velocity of choline transport in the ipsilateral fascia dentata within 1 day. Studies conducted 5-104 days after operation showed a consistent 55-65% elevation (on a protein basis) in the denervated area. In contrast, choline transport in the granular layer was similar on operated and control sides. Treatment of the rats immediately before sacrifice with pentylenetetrazole or pentobarbital, drugs previously shown to raise or lower choline transport by drastically altering impulse flow in the septohippocampal fibers, failed to abolish the lesion-induced increase, suggesting that removal of the perforant path fibers did not elevate choline transport solely by increasing the endogenous firing rate. Calculation of choline transport on a whole regional, rather than protein, basis revealed that fasciae dentatae from operated and control sides transported choline at approximately equal rates, but on the operated side a greater percentage was transported by structures from the molecular layer and a lesser percentage by those from the granular layer. The changes in high affinity choline transport coincided spatially and temporally with the reactive growth of septohippocampal fibers. Our results suggest that septohippocampal fibers react to perforant path lesions during development by rapidly establishing more synapses than normal in the denervated area at the expense of deeper layers and that the anomalous presynaptic elements persist into adulthood. (Supported by NSF grant BNS76-09973).

1381 DEVELOPMENT OF FUNCTIONAL RETINOTECTAL CONNECTIONS IN CULTURES OF EMBRYONIC MOUSE EXPLANTS. Neil R. Smalheiser*, Stanley M. Crain and Murray B. Bornstein. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Explants of retina and whole superior colliculi (tecta) from 13- and 14-day mouse embryos were cultured on collagen-coated coverslips in Maximow slide chambers for up to 6 weeks. Retinal explants developed characteristic "rosettes", and laminar arrays of neurons and glia (e.g. LaVail and Hild, Z.Zellf. 114:557,'71). Small isolated fragments showed diffuse neuritic outgrowth, whereas in explants of the whole eyeball, large single optic nerves emerged within the first few days after explantation. Recordings of individual retinal or tectal explants (with 3-5 μ electrodes) showed organotypic spontaneous and electrically-evoked repetitive spike and slow-wave patterns, with characteristic pharmacologic sensitivities to d-tubocurarine, strychnine, and bicuculline (10^{-6}-10^{-5}M) (Smalheiser, Crain and Bornstein, Anat. Rec. 187:780,'77). Preliminary data from two eyeballs maintained for several weeks in vitro indicate that a spike-barrage response may occur at a long latency (1-2 sec) after a light flash, but no response to light was seen in isolated retinal explants. In retinotectal co-cultures, single focal stimuli in the retinal explants (with 5-10 μ electrodes) evoked characteristic tectal responses at stimulus strengths much lower than needed to backfire tectal neurites. Stimuli applied to retinal neurites in the outgrowth far from the tectum also evoked similar tectal responses. The threshold to elicit tectal responses was low only within a narrow zone of depth in the retinal explants, similar to the optimal depth for recording retinal spike activity. These data, and other controls, indicate that functional retinotectal connections developed in vitro.

Microelectrode mapping is being used to trace growth of retinal neurites into the tectal explant, and to see if they may project to specific regionally localized sites in explants of the whole tectum (cf. sensory target zones in DRG-cord-medulla explants: Crain and Peterson, Science 188:275,'75). Electrophysiologic analyses in co-cultures of retinal pieces with whole tecta suggest that groups of individually characterized axons arising from ganglion cells in a small region of the retinal explant tend to arborize within a restricted zone of the tectal explant, while axons from another retinal locus tend to be associated with a different tectal zone. Topographic mapping of whole, oriented explants of retina and tectum is currently being pursued, both with electrophysiologic and peroxidase transport techniques.

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1382 EFFECTS OF NEONATAL VISUAL CORTEX DAMAGE ON LATERAL SUPRASYLVIAN VISUAL AREA NEURONS: EVIDENCE FOR FUNCTIONAL COMPENSATION. Peter D. Spear and R. E. Kalil. Dept. of Psychology and Dept. of Anatomy, University of Wisconsin, Madison, Wisc. 53706.

Visual cortex damage in kittens produces less severe behavioral deficits than similar damage in adult cats. Indeed, the kittens may develop normal form and pattern vision in spite of the lesion. Anatomical experiments indicate that an unusually heavy projection develops from retina to thalamus to the lateral suprasylvian visual cortex (LS area) in such animals (Kalil, unpublished). In addition, there is behavioral evidence that the LS cortex is involved in the recovery of form and pattern vision following visual cortex damage in adult cats (Baumann & Spear, Brain Res., 1977, in press). These findings led us to investigate whether neonatal visual cortex damage results in altered development of the functional properties of LS cortex neurons.

Kittens received unilateral visual cortex damage (areas 17, 18, and 19) when they were one day old, and single unit recording was conducted in LS cortex when they were 7-8 months old. The receptive field properties were compared with the properties of LS cortex cells in normal cats (Spear & Baumann, J. Neurophysiol, 1975, 38:1403) and in cats with unilateral visual cortex damage incurred as adults (Spear & Baumann, Neurosci. Abs., 1975, 1:62). In normal cats, 81% of the LS cortex cells are direction selective, and most (65.5%) of the cells are binocularly driven. Following unilateral removal of visual cortex in adults, there is a marked reduction in the percent of cells with direction selective receptive fields (21.5%), and an increase in the effectiveness of stationary flashing stimuli among the non-direction selective cells. In addition, the majority of cells (56.5%) now are driven only by the contralateral eye.

The effects of neonatal visual cortex damage are very different. The receptive fields of LS cortex cells appear normal in these animals--in spite of the absence of visual cortex. Most (74.5%) of the cells are direction selective, and their directional tuning is normal. The cells respond poorly to stationary flashing stimuli, just as in normal cats. The percent of binocularly driven cells (66%) also is normal. Thus, there is a functional compensation in LS cortex following neonatal visual cortex damage. It appears that the LS cortex cells use the anomalous retino-thalamo-cortical inputs to develop receptive field properties normally provided by the missing visual cortex inputs.

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1383 CELL PROLIFERATION IN THE "COMPRESSING" OPTIC TECTUM OF GOLDFISH. James A. Stevenson. Dept. Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada.

The phenomenon of visual field compression in the goldfish tectum has been demonstrated repeatedly by a series of investigators using electrophysiological, anatomical, and behavioral methods. The present experiments look at morphological changes within the tectum which accompany compression. Time series studies of cell proliferation in the halved tectum were carried out to explore the possibility that the birth of new tectal cells might induce or permit the alteration in the pattern of functional connectivity which is obtained during compression.

Sixteen goldfish received bilateral optic nerve crush and unilateral excision of the caudal half of the tectum. At five day intervals from 5 to 40 days after the operation pairs of fish were injected with tritiated thymidine and were sacrificed two days after labelling. Labelling in both the halved tecta and the contralateral control tecta was similar up to 20 days after surgery. Increased numbers of labelled cells were seen in the halved tecta of fish labelled at 25 and 35 days after surgery. This labelling was seen primarily in those tectal zones which receive direct retinal input. In addition, there was a slight increase in the number of labelled periependymal cells in the halved tecta of these fish. We have observed that these periependymal cells proliferate during tectum reinnervation.

In a second series of fish which were operated and labelled in a similar manner, but which were allowed longer post-labelling survival times, labelled cells were still seen in all tecta but appeared in equal numbers in halved and control tecta.

These results indicate the existence of a minor degree of cell proliferation during reinnervation which is augmented by half-tectum excision. It is possible that this additional proliferation is a consequence of degenerative processes which accompany the fiber and axon terminal shuffling which is required during the transition from a normal to a compressed projection pattern. The results do not support the notion of a massive cell proliferation which reconstitutes, in volume or in number, a full neuronal complement to a surgically halved optic tectum.

1384 THE PHYSIOLOGICAL EFFECTS OF MONOCULAR DEPRIVATION IN VERY YOUNG KITTENS. Richard C. Van Sluyters* and Ralph D. Freeman. School of Optometry, Univ. of California, Berkeley, Ca. 94720

Recent studies conducted in our laboratory have led us to believe that coordinated binocular vision may occur very early in a kitten's life. Optical quality and interocular alignment seem adequate to support binocular vision and neonatal surgical strabismus apparently has a disruptive effect. Given these findings, it might be expected that monocular deprivation in very young kittens would cause a loss of cortical input from the deprived eye just as it does in older kittens. To investigate this possibility, we have studied the receptive field properties of cells recorded from the striate cortex of two groups of kittens ranging in age from 9 to 23 days. In a control series of normally reared kittens, the vast majority of visually responsive neurons was binocularly driven. A second group of kittens was reared with monocular lid suture beginning at 9 days and lasting for from 6 to 14 days. The cortical ocular dominance patterns of these kittens showed a marked shift in favor of the input from the nondeprived eye. We conclude that the physiological properties of cells in the kitten's visual system can be extensively modified by altered visual experience during the first 3 weeks of life.

1385 EFFECTS OF Δ^9-THC ON FREQUENCY POTENTIATION AND RECURRENT INHIBITION IN RAT HIPPOCAMPUS. D. J. Weisz*, D. L. Gunnell* and R. M. Vardaris. Dept. of Psych., Kent State Univ., Kent, OH 44242.

The effects of Δ^9-tetrahydrocannabinol (THC) on CA1 field potentials have been previously investigated in paralyzed, locally anesthetized rats. Intraperitoneal administration of 2, 4, and 8 mg/kg THC resulted in an enhancement of spike amplitudes at 30-45 min post-injection but a depression at 120-150 min. A dose of 16 mg/kg THC depressed spike amplitudes at 30-150 min. Latencies to peak amplitude increased following THC administration. In the present investigation we examined the effects of THC on low frequency potentiation (LFP) of CA1 field potentials and on the extent and time course of recurrent inhibition in the CA1 area.

Following recovery from surgical anesthesia, recordings were made from locally anesthetized, paralyzed rats. Stimulation of the Schaffer collateral branch of the CA3 axons elicited extracellular population EPSP's and spikes from the CA1 pyramids. After a stable baseline was obtained, the CA1 population spikes were potentiated by presenting 10 stimuli at 1/sec. Then a dose of 0, 2, 4, 8, or 16 mg/kg THC was administered i.p. and LFP was produced in the CA1 pyramids at 30, 60, and 90 min after drug injection. Prior to each LFP the stimulus intensity was adjusted so that spike amplitudes were within ±.75 mV of predrug baseline. Potentiation of the CA1 population spikes was attentuated in a dose-dependent manner following administration of THC. The effect was maximal at 60 min postdrug. A dose of 16 mg/kg THC almost completely blocked potentiation at 60-90 min.

In a second study the effect of THC on recurrent inhibition was examined. The stimulus intensity eliciting maximal population spikes was used. Conditioning-test (C-T) shock intervals were varied from 20- 2000 msec. Predrug data showed that inhibition was present with 20-200 msec C-T intervals. Following the initial C-T series an i.p. dose of 0, 2, 4, 8, or 16 mg/kg THC was given. In a dose-dependent manner THC increased the range of C-T intervals over which recurrent inhibition was effective, such that after 16 mg/kg THC measurable inhibition occurred at an interval of 500 msec. While increased inhibition was observed following THC at C-T intervals of 60-300 msec, there was a tendency for disinhibition to occur at a C-T intervals of 20 msec.

1386 CHANGES IN THE ABSOLUTE VOLUMES OF THE TERMINAL FIELDS OF FIBER SYSTEMS PROJECTING TO THE FASCIA DENTATA AFTER SELECTIVE DESTRUCTION OF THE MEDIAL PERFORANT PATH. Mark J. West and Jens Zimmer*, Institute of Anatomy, University of Aarhus, 8000 Aarhus C, Denmark.

Discrete unilateral lesions were placed in the medial entorhinal cortices of one day old rats, resulting in partial destruction of the medial perforant paths (MPP) to the fasciae dentatae. Previous semi-quantitative evaluations of this type of preparation have indicated that the terminal fields of fiber projections to the zones adjacent to the MPP zone (i.e. lateral perforant path (LPP) and ipsilateral-commissural hippocampal-dentate projection (IC)) occupy a larger proportion of the mature fascia dentata than they do in normal animals. However, the absolute nature of this reorganization, which may also involve the residual MPP itself, has not been clear, in that it is not known whether the terminal fields are larger or smaller than those in the normal fascia dentata.

The absolute volumes of the terminal fields of the fiber systems projecting to the fascia dentata, as defined in Timm-sulfide silver stained preparations, have been determined with the aid of a small digital computer. A preliminary comparison of the terminal fields in the deafferented fascia dentata, with those in the contralateral control fascia dentata, indicates that the absolute volume of the terminal field of the MPP is smaller, and those for both the LPP and IC are larger, on the deafferented side. The total volume of the combined terminal fields is the same on both sides, however, suggesting that the total synaptic and dendritic volume is the same in the control and deafferented fasciae dentatae.

PSYCHOPHARMACOLOGY

1387 EFFECT OF AMINO ACID MANIPULATION ON RAT SHUTTLE AVOIDANCE PERFORMANCE.[1] Perrie M. Adams, Frnest S. Barratt, Richard R. Fritz*, Phil L. Poffenbarger* and Creed W. Abell*. Depts. of Psychiatry, Human Biological Chemistry and Genetics and Internal Medicine, University of Texas Medical Branch, Galveston, Texas 77550.

Performance of shuttle avoidance was studied in groups of rats following injection of the enzyme phenylalanine ammonia-lyase (PAL). This enzyme rapidly deaminates plasma phenylalanine and tyrosine with the resulting reduction of these brain amino acids and of Dopamine and Norepinephrine at 8 to 24 hours post-injection. Brain tryptophan and brain serotonin are significantly elevated for up to 8 hours after the enzyme administration. Groups of rats were studied at 4 or 24 hours following PAL injection or following a phosphate-buffer control injection. Performance of the 4-hour animals was significantly impaired as determined by fewer avoidance responses, slower avoidance trial latencies and slower escape trial latencies. Performance of the 24-hour or phosphate-control animals were not statistically different from each other for any of these measures. Biochemical assays on the brains of these animals confirmed the elevation of serotonin in the 4-hour group and the depletion of brain catecholamines in the 24-hour post-PAL group. These results support previous findings which suggest a serotonin mediated inhibitory mechanism. In addition, these findings support the significance of amino acid imbalance in the production of behavioral changes.

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1388 DOPAMINE-β-HYDROXYLASE INHIBITORS (DBHI) REVERSE THE EFFECTS OF NEUROLEPTICS UNDER ACTIVATING CONDITIONS: POSSIBLE EVIDENCE FOR A NOREPINEPHRINE (NE)-DOPAMINE (DA) INTERACTION. Seymour M. Antelman* and Cynthia A. Black* (SPON: K.R. Carlson). Dept. of Psychiatry and Psychobiology Program, University of Pittsburgh, Sch. Med., Pittsburgh, Pa. 15260.

We have recently proposed that a stress-dependent interaction exists between NE and DA (ANTELMAN and CAGGIULA, Science, Vol. 195, pp. 646-653, 1977). This hypothesis predicts that the attenuation of certain types of behavior typically seen following interference with DA function is reversible by additionally interfering with NE function under stressful but not quiescent conditions. As a test of this hypothesis we studied the effects in rats of the DA-receptor antagonist, haloperidol, when administered alone or with one of two NE-synthesis inhibitors (DBHI's), FLA-63 or Methimazole on both stress-related eating (induced by tail-pinch) or relatively unactivated, ad-lib feeding.

The results of these experiments clearly indicated that haloperidol (at 0.2 or 0.4 mg/kg, i.p.) attenuated both tail-pinch-induced eating and ad-lib feeding (during a four-hr. test). However, while both FLA-63 (at 10 and 20 mg/kg, i.p.) and methimazole (at 25 and 50 mg/kg, i.p.) significantly reinstated tail-pinch-induced eating when paired with haloperidol, they had no effect whatever in reversing haloperidol suppression of eating in the non-stress, ad-lib situation. These results support the hypothesis that stress-related interaction exists between NE (although some other β-hydroxylated amine cannot yet be ruled out) and DA.

Tail-pinch behavior appears importantly dependent on the nigrostriatal DA pathway which seems to be reciprocally related to striatal acetylcholine (Ach). It therefore seems likely that the effects of neuroleptics on tail-pinch behavior may, in part be due to an imbalance between DA and Ach in favor of the latter. This suggests the possibility that the ability of DBHI's to counteract neuroleptic effects during activating circumstances may be due to an influence of these agents (directly or indirectly) on striatal Ach. As a preliminary test of this hypothesis, we first determined that the anti-cholinesterase inhibitor, physostigmine (0.5 mg/kg, i.p.) would attenuate tail-pinch-induced eating and then tested it on this same paradigm in combination with FLA-63 or methimazole. Just as occurred with haloperidol, both DBHI's significantly reversed the attenuating influence of physostigmine on stress-induced eating.

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1389 AN EVALUATION OF THE EFFECTS OF PSYCHOTOMIMETIC DRUGS ON THE CONDITIONED EMOTIONAL RESPONSE IN RATS. John M. Beaton, Wu-Fuu Liu* and Robert S. Teague*, Neurosci. Prog. and Dept. of Pharmacol. and Psychiat., Univ. of Alabama in Birmingham, AL 35294.

It has been suggested that the disease schizophrenia may be caused by either the production, or the increased production, of a methylated psychotoxin e.g. N,N-dimethyltryptamine (DMT) or O-methyl-bufotenin. Christian et al. (Ala. J. Med. Sci., 13: 162, 1976) have shown that DMT occurs in the brain of rats and in human cerebral spinal fluid. These workers have also shown that shock stress can increase the rat brain levels of DMT approximately four-fold. This finding is of interest because it has been shown that environmental stress precipitates the hospitalization of approximately 50% of schizophrenics (Beck and Worthen, Arch. Gen. Psychiat., 26: 123, 1972).

The present study was carried out to examine the effects of various compounds, d-amphetamine, DMT, mescaline and two other agents with possible anti-DMT effects, on rat behavior before and after the addition of shock stress, via the superimposition of a conditioned emotional response (CER). Eleven, male adult Long-Evans rats were trained on a variable interval (VI) 30 second schedule of reinforcement. After initial testing of each dosage of each drug in all eleven animals the CER schedule was superimposed. This consisted of eight pairings of one minute of tone followed by a brief unavoidable shock, per session. The compounds were tested again at each dose level after the CER was well established. The data for rate of bar pressing and number of reinforcements obtained for the pre-CER were compared with the CER data.

Paired-t tests showed that the CER significantly potentiated the behavior disrupting effects of amphetamine (1.0 mg/kg), mescaline (10.0 mg/kg) and DMT (5.0 mg/kg). Neither of the postulated anti-DMT agents, N,N-diethylbutyramide (DBA) and 1-methyl-1,2,5,6-tetrahydropyridine-3-N,N-diethyl-carboxamide (THPC), which had previously been shown to block the disruption of DMT of avoidance behavior blocked the behavior disruption of DMT on this VI 30 sec schedule of positive reinforcement. However, this study has shown that the addition of shock stress via the CER schedule potentiated the disruption of the hallucinogens mescaline and DMT, and of the stimulant amphetamine. The mode of action of this potentiation, i.e. whether or not the stress led to an increase in endogenous DMT, can not be determined from this study. However, it would be of interest to examine the brain levels of DMT in rats exposed to a similar CER schedule.

1390 INTERACTIVE EFFECTS OF LITHIUM AND d-AMPHETAMINE ON THE ACOUSTIC STARTLE RESPONSE IN THE RAT. Michael S. Beattie and Donald R. Britton*, Depts. of Anat. and Pharmacol., College of Medicine, The Ohio State University, Columbus, Ohio 43210.

Lithium administration (2 meq/kg/day for 6 days) has been shown to result in attenuation of amphetamine or environmentally induced increases in locomotor activity. The present study investigated the effects of lithium and d-amphetamine on sensorimotor reactivity using the acoustic startle reflex (ASR) as a measure of responsiveness.

Male hooded rats, housed in groups of 3, were injected i.p. with 2 meq/kg lithium chloride (LiCl) on each of the 6 days preceeding ASR testing. Control animals received 2 meq/kg saline. ASR testing consisted of 50 presentations of a 105 db, 2500 Hz tone given once every 60 sec. 6 LiCl and 6 saline treated rats were tested to assess the effects of lithium on ASR magnitude and habituation. 7 additional animals from each treatment condition were given i.p. injections of d-amphetamine sulfate (2.5 mg/kg, salt wgt) 5 min. prior to testing.

Initial response magnitudes, as well as the degree of response decrement over the session, were very similar for rats treated with LiCl or saline. Amphetamine given to saline treated animals produced a marked potentiation of ASR magnitudes over the course of the session, a result consistent with previous studies (Davis et al, 1975). However, amphetamine failed to produce increased responsiveness in animals treated with LiCl. Thus, while lithium had no apparent primary effect on reactivity at the dosage and schedule employed such treatments were effective in blocking the potentiation of reactivity induced by a 2.5 mg/kg dose of d-amphetamine. These results lend support to the notion that the behavioral actions of lithium may be mediated, in part, via the actions of this substance upon catecholamine systems.

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1391 A NEW ANIMAL MODEL FOR SCHIZOPHRENIA: ADRENERGIC MODULATION. R.L. Borison*, H.S. Havdala* and B.I. Diamond* (SPON: V. Nair). Illinois State Psychiatric Institute and Anesthesiology Department, Mount Sinai Hospital, Chicago, IL 60612 and 60608.

Previously we have proposed phenylethylamine induced stereotyped behavior as a new animal model for schizophrenia because phenylethylamine elicited stereotypy is more sensitive than d-amphetamine induced stereotypy to blockade by antipsychotic agents. Phenylethylamine is an endogenous amphetamine-like central nervous system stimulant which is most highly concentrated in the limbic system of human brain, and phenylethylamine urinary excretion has been reported as abnormal in schizophrenic subjects. We have previously shown that chronic phenylethylamine (50 mg/kg) induced stereotypy is qualitatively and quantitatively similar to chronic d-amphetamine (3.75 mg/kg) elicited stereotypy, but that phenylethylamine induced stereotypy is preferentially blocked by the antipsychotic agents clozapine and thioridazine (Borison et al, Life Sci., in press). We now report the effects of various adrenergic agents upon both phenylethylamine and d-amphetamine induced stereotypy. Pretreatment with the α-adrenergic blocking agents phentolamine (45 mg/kg) or phenoxybenzamine (20 mg/kg) prevented phenylethylamine behavior, whereas d-amphetamine stereotypy was only partially antagonized. In contrast, propranolol (20 mg/kg), a β-adrenergic receptor blocker, reduced slightly phenylethylamine's actions while potentiating those of d-amphetamine. The catecholamine depletor α-methyl-para-tyrosine (250 mg/kg) antagonized the stereotypies induced by either phenylethylamine or d-amphetamine. When brain norepinephrine was depleted by administration of diethyldithiocarbamate (20 mg/kg), there was a blockade of phenylethylamine behavior and an augmentation d-amphetamine evoked stereotyped behavior. Studies with the aminoacid precursor to norepinephrine, dihydroxyphenylserine (400 mg/kg), showed that both phenylethylamine and d-amphetamine stereotyped behavior was potentiated. The aminoacid precursor to dopamine, L-dihydroxyphenylalanine (50 mg/kg), antagonized both phenylethylamine and d-amphetamine stereotypy. These results suggest that the stereotypies induced by chronic phenylethylamine may be mediated via noradrenergic as well as dopaminergic systems, while d-amphetamine induced behavior is most likely mediated through dopaminergic systems.

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1392 DOSE DEPENDENT PSYCHOLOGIC AND NEUROENDOCRINE EFFECTS OF DEXTROAMPHETAMINE AND METHYLPHENIDATE. Walter A. Brown, Prov. VA Hospital and Brown University, Prov., R. I. 02908.

Dextroamphetamine (DA) and Methylphenidate (MP) have well documented effects on the disposition of central catecholamines and have general arousing and euphoric effects. DA has been shown to stimulate release of growth hormone (GH) and cortisol while MP stimulates GH release but not cortisol release. This study is directed toward identifying the dose response characteristics of the various psychologic and endocrine effects of DA and MP and examining the relationships among the psychologic and endocrine effects. Clarification of these relationships could provide a step toward elucidation of the central mechanisms mediating the psychologic effects of DA and MP.

49 healthy young adult men (mean age 24) ingested identical capsules containing either 20 mg DA, 10 mg DA, 20 mg MP or 10 mg MP on a double blind basis. Blood samples drawn during baseline and at 15 min. intervals for 2 hours after drug ingestion were assayed for GH, cortisol, and amphetamine (A), by radioimmunoassay. Before and 90 mins. after drug ingestion subjects completed the Nowlis Mood Adjective Check List (MACL) scored for 7 scales related to anxiety, activation and euphoria and 4 scales of the Addiction Research Center Inventory (ARCI) related to the psychologic dimensions of arousal and euphoria.

Dose (mg/kg) of DA was significantly correlated with peak serum A concentration ($r = .60$; $p = .002$) with increment in serum GH ($r = .60$; $p = .002$) and with increase on the ARCI euphoria scale ($r = .48$; $p = .009$). Dose of MP was significantly correlated with increase on an MACL euphoria scale ($r = .41$; $p = .027$) and showed a trend toward positive correlation with GH response ($r = .32$; $p = .092$). Increment in serum cortisol and psychologic changes along dimensions of activation and arousal were not dose dependent. GH response to both DA and MP was selectively correlated with responses on several scales related to the general dimension of euphoria while cortisol response was positively correlated with responses on scales measuring both arousal and euphoria.

These data suggest that dose of DA and MP is selectively related to the GH and euphoric responses. These two responses may be mediated by a central neurophysiologic process which differs from that which mediates the cortisol response and general arousal. The selective dose dependency of GH and euphoric response and their positive correlation points to a common neurochemical process, possibly involving dopamine, mediating these psychologic and neuroendocrine events.

1393 DISSOCIATION OF ACTIVE FROM PASSIVE COMPONENTS OF SEXUAL BEHAVIOR IN FEMALE RATS BY DESTRUCTION OF BRAIN CATECHOLAMINE-CONTAINING NEURONS. Anthony R. Caggiula*, James G. Herndon, Jr.*, Robert Scanlon*, Deborah Greenstone*, Wilson Bradshaw* and Donna Sharp*. Psychobiology Program, Dept. Psychology, Univ. of Pittsburgh, PA 15260 and Yerkes Primate Center, Emory Univ., Atl. GA 30322 (SPON: M. Alexander).

We have recently reported (Herndon et al., 1976) that an intraventricular 6-hydroxydopamine (6-OHDA) treatment, which produced moderate depletions of telencephalic norepinephrine (NE) and dopamine (DA) (66% and 56% respectively) resulted in moderate increases in the frequency and intensity of lordosis (the passive component of sexual behavior) in females given low doses of estradiol and tested with male rats. This effect could be markedly intensified by the addition of the CA synthesis inhibitor, α-methyl-p-tyrosine (100 mg/kg). However, neither condition resulted in the display of soliciting behavior (the active component). These results, suggesting differential effects of CA depletion on the active and passive components of female sexual behavior, have been substantiated and extended as follows.

First, ovariectomized female rats were given a hormone treatment (8 μg/kg estradiol benzoate) which normally supports only low levels of lordosis responding and no soliciting behavior. When subjected to an intraventricular 6-OHDA regimen (with pargyline pretreatment) which produced a more substantial CA lesion (85% and 95% depletions of caudate DA and cortical NE respectively), these females exhibited a 300-350% increase in the intensity and frequency of lordosis behavior, but absolutely no soliciting behavior over the 3 weeks of testing.

Second, in females given a hormone regimen (50 μg/kg estradiol benzoate plus 500 μg progesterone), which supported maximal levels of lordosis and soliciting, the same 6-OHDA treatment prolonged the average duration of lordosis while actually decreasing the incidence of soliciting behavior.

Third, we addressed the question of whether the increase in receptivity that was obtained by 6-OHDA might be due to a drug-induced release of adrenal progesterone. Experiments utilizing dexamethasone suppression of adrenal function, documented by RIA measurement of circulating progesterone, clearly show that the dramatic increase in lordosis responding produced by 6-OHDA was not associated with any measurable release of progesterone.

These results suggest an important refinement in current hypotheses relating brain CA activity to global behavioral categories such as "male" or "female" sexual behavior, and, more generally, in hypotheses dealing with the role of CA systems in sensorimotor responsiveness.

1394 RED CELL BOUND BUTAPERAZINE AND THERAPEUTIC RESPONSE IN SCHIZOPHRENIA. R.C. Casper, D.L. Garver, S. Chang, S. Ericksen, H. Dekirmenjian, J.M. Davis. Ill. State Psychiatric Inst., & Dept. Psychiatry, Abraham Lincoln School of Med., Chicago, IL 60612.

We report data relating plasma and red blood cell (RBC) butaperazine (BPZ) to therapeutic response in 23 schizophrenic and chronic schizoaffective patients during the first 2 weeks of steady dose treatment. Therapeutic response was monitored by % change in serial modified New Haven Schizophrenic Indices during the 2 week period. Mean plasma and mean RBC BPZ from day 4 through 14 of treatment was computed from data based on the assays according to the method of Dekirmenjian. Multiple curve fittings relating plasma and RBC BPZ separately to % therapeutic response revealed that the best fit was to a quadratic polynomial for both plasma ($r^2 = .267$) and RBC ($r^2 = .597$) BPZ. RBC BPZ fit significantly better ($z = 1.99$; $p<.05$) than did plasma BPZ to therapeutic response data. The data demonstrated a "therapeutic window" for blood levels of BPZ, both above which and below which schizophrenic symptoms are less well controlled by BPZ. Moreover, therapeutic response correlates better to levels of BPZ bound to the RBC than to the BPZ in plasma. RBC BPZ may better reflect quantities of drug which are effective in regulating the activity of central nervous system dopamine receptors.

1395 INVESTIGATION FOLLOWING ACUTE AND CHRONIC D-AMPHETAMINE TREATMENT IN THE GERBIL. MaryLou Cheal. Neuropsychology Laboratory, McLean Hospital, Belmont, MA 02178.

Interest in environmental stimuli often appears inappropriate in humans with amphetamine psychosis and in animals given large doses of amphetamine. Although the stereotypic behaviors elicited with amphetamine have been described as evolving from investigative behavior (Ellinwood, Sudilovsky, & Nelson, Am. J. Psychiatry, 1973, 130, 1088-1093), these fragments of normal behavior patterns become abnormally repetitious. Rats have been noted to persistently sniff at a particular area; cats move their heads as if looking around; and monkeys develop more complicated movements, coordinating hand and eye. Such behaviors often involve excessive grooming movements. In our laboratory, gerbils emit a repetitious sequence of paw licking and face washing. It is not known whether these investigatory behaviors are internally generated and not related to the stimulus, or whether the animal becomes more responsive to the object of investigation.

To answer the question, gerbils were chosen because of their extremely active, investigatory nature, and were presented with either a novel object or a socially relevant odor within a small enclosure. Following treatment with low acute (0.5-2.0 mg/kg) or chronic (0.5 mg/kg) doses of d-amphetamine or NaCl, gerbils displayed normal levels of investigation and subsequent habituation to either the object or the odor. If the object was moved to the opposite side of the arena following habituation, investigation was again elicited. Moving the odor did not renew interest. Acute treatment with 3.0 mg/kg d-amphetamine induced stereotypies in all animals and the amount of investigatory time was significantly attenuated. However, there was still significant habituation, and a significant increase in investigation when the object was moved. We conclude that, in the gerbil, amphetamine does not cause increased responsiveness to stimuli. In fact, gerbils respond selectively to specific stimuli in spite of the probability that internally generated amphetamine-induced stereotypies are competing with stimulus-elicited investigation.

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1396 ACETALDEHYDE MEASUREMENTS FROM BREATH SAMPLES. H. Dix Christensen, Arthur Zeiner and Jane Drake*. Depts. Pharm., Med., and Psychiat. and Behav. Sci. Univ. of Okla. Health Sciences Center, Oklahoma City Okla. 73190.

Alveolar air concentrations of acetaldehyde should essentially reflect the biotransformation of acetaldehyde from ethanol. For many ethnic studies multiple samples need to be collected in the field. Acetaldehyde and ethanol levels were compared in breath directly and by a trapping procedure developed by Dr. Pellizzari of the Research Triangle Institute. Six fasted subjects drank within ten minutes 0.66 ml/kg of ethanol as a 20% solution in orange juice. A series of alveolar air samples were collected over three to five hours by having the subject exhale deeply at the end of normal expiration through a teflon tubing into a 25 ml serum bottle. The internal standard consisted of 12.5 ng isobutyaldehyde. For analysis one ml of headspace was used either directly or after trapping with 100 µl of 0.04M sodium metabisulfite and later released by heating to 60°C for 50 minutes with 100 µl of 0.25M "Clark and Lub's Borate Buffer" pH 8.5. There is about 65% recovery of acetaldehyde after trapping and release. Acetaldehyde and ethanol concentrations were determined by a gas chromatograph method using a Hewlett-Packard Model 402 gas chromatograph with a hydrogen flame detector. The 6 ft long, 1/8 inch i.d. glass column was packed with Porapak P 100/200 mesh. The operating temperatures were: column 110°, detector 210°C and injector port temperature 150°C. Retention times were methanol 1.6, acetaldehyde 2.2, ethanol 3.2, acetone 5.4 and the isobutyraldehyde 10.0. Elimination of moisture is critical for the direct breath method. The trapping method is effective for acetaldehyde but not ethanol.

Ethanol concentration in alveolar air increased rapidly with a $t_{1/2}a$ of 15 ± 1 minutes, reaching a peak of 32 ± 3 µg/100 ml at 37 ± 4 minutes and declined linearly with a $t_{1/2}e$ of 122 ± 7 minutes. Acetaldehyde kinetic parameters were $t_{1/2}a$ of 18 ± 1 minutes with a plateau concentration of 475 ± 18 ng/100 ml from 41 ± 3 minutes to 119 ± 14 minutes then with a $t_{1/2}e$ of 205 ± 32 minutes. Zero time was the start of drinking.

Acetaldehyde plateau levels are apparently a function of the metabolism of the individual rather than the ethanol levels. Freund and O'Hollaren (J. Lipid Res. 6 471, 1965) reported similar results of acetaldehyde rise, plateau and then decline when ethanol concentrations return to 15-25 µg/ml alveolar air range. The trapping-release method permits analysis of acetaldehyde up to a month from time of sample collection.

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1397 BEHAVIORAL, BIOCHEMICAL AND MORPHOLOGICAL CHANGES AFTER INTRASTRIATAL INJECTIONS OF KAINIC ACID (KA). B. R. Cooper*, R. M. Ferris* and H. L. White*. (SPON: Warren C. Stern). Dept. of Pharmacol., Wellcome Res. Labs, Research Triangle Pk., N.C. 27709

Intrastriatal injection of KA, a cyclic glutamate analogue, has been reported to produce biochemical changes characteristic of Huntington's chorea through a selective "excitotoxic" action on acetylcholine and GABA containing neurons, sparing dopamine terminals (McGeer & McGeer, Nature 263, 517-518, 1976). It was of interest to determine how prior treatment with KA would alter the response to dopamine agonists. In our initial experiments, the effects of apomorphine were compared to those of d-amphetamine after unilateral intrastriatal injections (1 µl) of KA (1 µg - 3 µg). Rotation ipsilateral to the injection occurred after treatment with both compounds when animals were tested 2 to 30 days after KA, suggesting lesion of cells containing DA receptors. Analysis of the injected (3 µg) versus contralateral untreated striata revealed choline acetyltransferase (ChA) and glutamic acid decarboxylase (GAD) were reduced by 60% and 85% respectively 48 hrs after KA. Tyrosine hydroxylase (TH) activity remained near control levels up to 5 days but then began to decrease by 8 days after treatment. Thirty days after treatment, GAD, ChA, TH activity, as well as weight of the injected striatum were greatly reduced. Similar, but less pronounced effects were seen at lower doses of KA (1 µg - 2 µg). Reduction of GAD, ChA and TH activity and striatal mass suggests a less specific neurotoxic action of KA than proposed by earlier investigations. The apparent specificity for GAD and ChA containing cells but not dopamine (TH containing) fibers up to 8 days after injection may reflect different time courses for degeneration of cells versus fiber terminals. While KA may not be a very "selective" neurotoxin, it is interesting to note that reduced striatal mass has been found in advanced cases of Huntington's disease.

1398 DISTRIBUTION OF BUTAPERAZINE BETWEEN ERYTHROCYTES AND PLASMA PROTEINS. H. Dekirmenjian, J.I. Javaid, D.L. Garver, S. Chang, J.M. Davis. Ill. State Psychiatric Inst., Chicago, IL 60612.

Recently we noted that red blood cell (RBC) levels of butaperazine (BPZ) correlated better with clinical response than did plasma levels (Am. J. Psychiat. 134:304, 1977). Most of the plasma drug is bound to blood proteins (95%-96%). Protein bound BPZ is ionized: thus, it is not soluble in lipids and will not pass the blood brain barrier. It would be expected that the free base, being soluble in lipids, will pass the blood brain barrier. If so, central nervous system drug levels will be in equilibrium with the free drug levels in the plasma. To investigate the rationale of why RBC levels should better reflect improvement in patients, in vitro experiments were carried out to determine whether RBC levels better reflected the free levels of the drug than the plasma. Human blood was obtained from control subjects in EDTA. RBC and plasma were separated and RBC washed 2 times with tris buffer. One ml of washed RBC and 1 ml of plasma were incubated with varying amounts of BPZ at 37°C for 5 min. RBC and plasma were again separated and BPZ determined in both of the fractions. The following experiments were carried out to observe their effect on BPZ binding to RBC: effect of time of incubation, increasing BPZ concentrations, varying pH of incubation buffer, effect of dilution of plasma, BPZ binding to bovine serum albumin, effect of dilution of bovine serum albumin, binding of the drug to RBC ghosts, and binding of the drug to rat synaptosomal membrane and rat RBC ghosts. From the above experiments, it could be shown that as the free BPZ levels increased in plasma so did the RBC bound BPZ. Thus there was an equilibrium between free drug levels & RBC levels. This was not a function of pH difference between plasma (pH = 7.4) and RBC (pH = 7.2) but was due to mass action of increasing levels of the unbound drug, in the plasma.

1399 MORPHINE INDUCED STEREOTYPY: IS IT DOPAMINE, NOREPINEPHRINE, OR ACETYLCHOLINE? B.I. Diamond*, H. S. Havdala*, F. Lemus* and R.L. Borison* (SPON: W. Harrison). Departments of Anesthesiology and Neurology, Mount Sinai Hospital, Chicago, IL 60608.

The chronic administration of morphine to rats produces tolerance to its behaviorally depressant effects and evokes hyperactivity and stereotyped behavior (Randrup et al, 1976). This stereotyped behavior mainly consists of oral self-directed behaviors, i.e. gnawing at paws, and is qualitatively similar to amphetamine induced stereotypy. Considering that psychoactive drugs capable of producing stereotypy, i.e. d-amphetamine and phenylethylamine, are believed to do so by affecting central dopaminergic mechanisms, we now report pharmacological studies aimed at better understanding the mechanisms underlying morphine stereotypy. We administered morphine (20 mg/kg) daily for three weeks to produce stereotypy. The latency to onset of stereotypy was five minutes and duration of stereotypy was greater than three hours. Stereotyped behaviors consisted of gnawing at the cage floor grid and paws, increased exploratory activity and hyperreactivity. We found that chronic morphine pretreatments sensitized animals to the stereotyped behavior induced by d-amphetamine (3.75 mg/kg) or phenylethylamine (40 mg/kg) (doses which are per se subthreshold for eliciting stereotypy). Moreover, acute pretreatments with d-amphetamine or phenylethylamine potentiated morphine evoked stereotypy. The antipsychotic neuroleptic dopamine blockers haloperidol (0.5 mg/kg) or pimozide (1 mg/kg) completely blocked morphine stereotypy, whereas neuroleptics with few extrapyramidal side-effects, clozapine (30 mg/kg) or thioridazine (50 mg/kg) increased to thirty minutes the latency to onset of morphine stereotypy. The cholinergic receptor blocking agent trihexyphenidyl (50 mg/kg) failed to alter morphine behavior, but in contrast when central acetylcholine levels were raised by pretreatment with physostigmine (0.5 mg/kg), there was a delay in onset and an antagonism to morphine stereotypy. When α-adrenergic receptors were blocked with phentolamine (45 mg/kg) there was an increase in latency to onset of stereotypy of one hour before normal morphine stereotypy was generated. Our results suggest that chronic morphine stereotyped behavior sensitizes adrenergic receptors in both the neostriatal as well as the meso-limbic system and is dependent, at least in part, upon central noradrenergic and dopaminergic mechanisms.

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1400 SELF-STIMULATION: EVIDENCE FOR TOLERANCE TO NEUROLEPTICS IN THE FRONTAL CORTEX. Alan J. Eichler*, Seymour M. Antelman* and Larry Kairns* (SPON: M. H. Bennett). Psychobiology Program, Dept. of Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260.

Since the discovery by Thierry, et al., (Brain Res., 50: 230, 1973) of a dopaminergic (DA) innervation of the cerebral cortex, this area of the brain has been considered as a possible site of action of anti-schizophrenic agents (neuroleptics). Biochemical evidence suggests that this area does not become tolerant to the effects of neuroleptics when they are administered for 11 days (Scatton, et al.,Brain Res., 109: 184, 1976). If borne out by longer term studies, these data would lend credence to a cortical DA hypothesis of schizophrenia, since tolerance does not develop to the antipsychotic properties of the neuroleptics.

We, therefore, re-examined the question of tolerance in the cortex of rats by determining the effects of long-term spiroperidol administration on electrical self-stimulation (SS) of the frontal cortex. Spiroperidol was given daily for 4 weeks in doses of 31,62 and 125ug/kg, and bar-press rates were observed both during drug administration and withdrawal. In contrast to results which we have previously reported for the nigrostriatal bundle (NSD; Eichler, et al., Neurosci. Abst., 2: 1225, 1976), 31 and 62ug/kg failed to depress frontal cortical SS when initially injected. Decreases in rate at these doses appeared only after 2-5 days of continued treatment. Approximately 2-3 weeks into the injection period, animals administered 31ug/kg began to increase their rate and were pressing at 140% of baseline by the time of withdrawal. At 62ug/kg, bar-pressing was virtually abolished from the 5th injection day until withdrawal. Animals receiving 125ug/kg did not press throughout the drug period. During withdrawal, however, significant rate increases (25-200%) at all doses occurred, which persisted unabated for at least 5 weeks.

The acute insensitivity of frontal cortical SS to doses of spiroperidol capable of producing severe depressions in SS from the NSD is consistent with the known relative DA turnover changes produced by neuroleptics in these systems. These data also argue against a performance deficit as totally responsible for neuroleptic-induced decreases in NSD SS.

The rate increase observed at 31ug/kg of spiroperidol during treatment, as well as the sustained withdrawal rate increases at all doses, indicate that tolerance to neuroleptics may occur in the cortical DA system of rat brain. The failure of previous reports to observe biochemical tolerance could be due to an inadequate period of neuroleptic administration. These results are at variance with the suggested involvement of cortical DA in the antipsychotic properties of neuroleptics, if such extrapolations can, indeed,be made from rat brain.

1401 ALTERATION IN MOTOR FREQUENCIES WITH DOPAMINE AGONISTS AND ANTAGONISTS. Everett H. Ellinwood, Jr. and M. Marlyne Kilbey. Dept. Psychiat., Duke Univ. Med. Ctr., Durham, NC 27710.

Although diseases of the extrapyramidal system are often expressed as changes in the intrinsic frequency of motor behavior (often tremor), little information exists in the literature on the frequency effects of drugs affecting the extrapyramidal and mesolimbic systems. This report will describe the differences in movement frequency in rats induced by dopamine agonists and antagonists. Movement is quantified with an electronic transducer and the analog output is analyzed and expressed as power spectrums. Changes in the power for specific frequencies provide a statistically significant dose-response curve for such drugs as apomorphine and amphetamine. Whereas traditional neuroleptics demonstrate a linear dose-response antagonism of amphetamine-induced frequency changes, clozapine induces a biphasic response. Differences in the alterations of specific frequencies may reflect a differential effect on mesolimbic vs. the striatal dopamine system.

1402 EFFECT OF CHRONIC L-DOPA ON APOMORPHINE-INDUCED ROTATION AND SNIFFING IN RATS. Cheryl Ezrin-Waters* and Philip Seeman. (Spon: Y. Israel). Pharmacology Department, University of Toronto, Toronto, Canada.

In order to study changes in dopaminergic brain sensitivity, the effect of chronic L-DOPA on apomorphine-induced behaviour was studied, using two types of tests.

1) L-DOPA (20 mg/kg) and carbidopa (2 mg/kg) p.o. were administered daily for 21 days to male Wistar rats. The animals received an acute injection of apomorphine (8, 16, 32 mg/kg i.p.) and the total number of rotations per minute were monitored over a 30 minute test period, during which six one-minute observations were made on each animal.

2) L-DOPA and carbidopa were given for 16 days to a second group of rats. Acute injections of apomorphine (0.5 - 2.0 mg/kg i.p.) resulted in sniffing behaviour and this was measured by direct observation.

After 2-3 weeks on the L-DOPA-carbidopa combination, many animals became tolerant to the apomorphine-induced turning. Preliminary results indicate that chronic L-DOPA alters the apomorphine-induced sniffing.

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1403 APOMORPHINE, BUT NOT CLONIDINE, REPAIRS DEFICITS IN LOCOMOTOR AND INVESTIGATORY EXPLORATION AFTER ABLATION OF DOPAMINE AFFERENTS TO LIMBIC FOREBRAIN AND ANTEROMEDIOVENTRAL STRIATUM. J. Stephen Fink and Gerard P. Smith. Dept. Psychiatry, Cornell Univ. Medical College and E.W. Bourne Behavioral Res. Lab., The New York Hospital, White Plains, N.Y. 10605

Bilateral injections of 6-hydroxydopamine (6-OHDA) in the anterolateral (AL) hypothalamus after pretreatment with desmethylimipramine (DMI, 25 mg/kg, i.p.) produces deficits in locomotor exploration (LE) in an open field (OF) and loss of dopamine (DA) fibers in mesolimbic, mesocortical and anteromedioventral striatal terminal fields (Neurosci. Abst. 2:454, 1976). After AL 6-OHDA-DMI injections there is little or no loss of catecholamine (CA) fibers in the norepinephrine (NE)-rich neocortex and hippocampus suggesting that DA loss within limbic forebrain and anteromedioventral striatum is sufficient, and NE loss in neocortex-hippocampus is not critical, for the deficits in LE. The present studies extend these observations to determine (1) if AL 6-OHDA-DMI injections produce deficits in investigatory exploration (IE) and (2) the effect of systemic administration of the DA agonist, apomorphine, and the α-adrenergic agonist, clonidine, on the exploratory deficits after AL 6-OHDA-DMI injections.

After AL 6-OHDA-DMI injections rats made fewer approaches to a novel object located in an alcove in the animals' home cages. After apomorphine (31-62 µg/kg, i.p.) AL 6-OHDA-DMI rats made more approaches to a novel object, but not to a familiar object or to an empty alcove. In AL 6-OHDA-DMI rats apomorphine (16-125 µg/kg, i.p.) increased LE in a novel, but not familiar, OF. The same doses of apomorphine decreased IE and LE in control rats. Because LE and IE after apomorphine in AL 6-OHDA-DMI rats is greater when the OF and object are novel than when they are familiar these responses are not simply increased random motor activity. Clonidine did not change (1 µg/kg) or decreased (100 µg/kg) IE and LE in both AL 6-OHDA-DMI and control rats.

We conclude (1) ablation of DA afferents to limbic forebrain and anteromedioventral striatum produces deficits in IE and LE, (2) NE afferents to neocortex and hippocampus do not appear to be critical to normal IE and LE in these tasks, (3) systemic administration of apomorphine after DA denervation of limbic forebrain and anteromedioventral striatum increases IE and LE to a novel, but not familiar, object and OF, and (4) DA afferents to mesolimbic, mesocortical and anteromedioventral terminal fields may subserve a permissive("arousal") function for LE and IE in the intact rat brain.

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1404 ENHANCEMENT OF L-TRYPTOPHAN-INDUCED DEPRESSION OF SYMPATHETIC PREGANGLIONIC NEURONS BY LITHIUM. Donald N. Franz* and Chaichan Sangdee* (SPON: D.M. Woodbury). Dept. of Pharmacology, Univ. Utah Col. Med., Salt Lake City, Utah 84132

Previous studies in cats indicate that 5-HT precursors depress the excitability of sympathetic preganglionic neurons (SPGNs), apparently by inducing spontaneous release of 5-HT from inhibitory bulbospinal 5-HT neurons (Neumayr, Hare & Franz, Life Sci. 14:793, 1974). This system was employed as a model to test the hypothesis that lithium enhances the uptake of L-tryptophan by central 5-HT neurons thereby increasing 5-HT synthesis (Knapp & Mandell, J. Pharmac. Exp. Ther. 193:812, 1975). In unanesthetized spinal cats, microelectrode stimulation of bulbospinal excitatory pathways in the dorsolateral funiculus of the cervical spinal cord at 10/min evoked brisk sympathetic discharges from SPGNs that were recorded from upper thoracic preganglionic white rami. Sizes of evoked discharges were analyzed every 5 or 10 min by signal averaging. Evoked discharges were gradually depressed to about 50% 3 hr after LiCl (2 mEq/kg), but this effect was prevented in cats depleted of 5-HT by pretreatment with PCPA. This indicated that lithium had no effect on the excitatory pathway. L-tryptophan (100 mg/kg) alone produced little or no depression. However, 3-4 hr after LiCl, this dose of L-tryptophan gradually depressed evoked discharges by about 30%. Onset was about 30 min and maximal depression was reached about 1 hr later and maintained for several hrs. After chronic LiCl pretreatment (1 mEq/kg twice daily for 3 days), L-tryptophan (100 mg/kg) rapidly depressed evoked discharges by about 60%; onset was about 10 min and maximum effect was established 30 min later and maintained for several hours. Plasma levels of lithium (8-10 hrs after last dose) ranged between 1.2 and 1.8 mEq/L (mean, 1.5). Acute lithium pretreatment had no detectible effect on depression after 5-HTP (30 mg/kg), but chronic pretreatment almost doubled the effect of this dose. These results support the proposal that lithium increases the uptake of L-tryptophan and 5-HTP by central 5-HT terminals and thereby enhances 5-HT synthesis.

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1405 POSSIBLE FELINE ANALOG OF TARDIVE DYSKINESIA. Robert B. Glassman, Harriet N. Glassman* and Christopher Frew*. Dept. Psychol., Lake Forest College, Lake Forest, IL 60045

Some psychotic patients who have been maintained on neuroleptic medication over a period of months to years develop a dyskinetic syndrome involving active movements of tongue and jaw. Considerable research has tended to localize the action of neuroleptic drugs at dopaminergic synapses in the corpus striatum (Snyder, et al., Science, 1974). Tardive dyskinesia may depend on changes in excitability there (Klawans, Amer. J. Psychiat., 1973) or elsewhere (Glassman, Behav. Sci., 1976). Cats are being prepared with chronically implanted gross electrodes at multiple subcortical and cortical loci, for recording of EEG and evoked potentials (EPs) over a period of months or years before, during, and after chronic administration of neuroleptics. In four animals so far we have seen increases in licking movements, "fly-chasing" movements of the skin of the back, and paw shakes, related to the drug treatment. These animals were observed during a three-month baseline period before being placed on chlorpromazine (25-35 mg/day orally). In the clearest case, the average number of licks was 10.8 per five-minute observation interval, taken on 13 occasions during baseline. During one month on chlorpromazine this average dropped to 6.7 licks. Following withdrawal, this animal has maintained a rate of approximately 233 licks per five minute period for a week, to date. Two other animals exhibited increased licking transiently after withdrawal. A fourth began to increase licking during the period of drug administration after 1½ weeks (6.3 licks/5' before vs. 189 licks/5' during). Additional data from other animals suggest that in some cases there may be a permanent increase in licking following longer maintenance on drug. Some of the licking behavior includes a wide opening of the mouth and lip licking; most becomes integrated into grooming behavior. To date, we have seen no consistent correlation across subjects between licking and EEG or EPs, but some individual cases show changes from baseline which appear linked to the drug treatment. For example, in one animal EPs recorded at several loci in basal ganglia (non-noxious shocks to forelimb in unanesthetized animals) increased only during drug treatment (300µV to 600 µV, at one point, negative, 5 msec. latency). In another case, a late, slow component of the sensorimotor cortical evoked potential was lost when the animal was placed on the drug and has not reappeared during 5 days since withdrawal.

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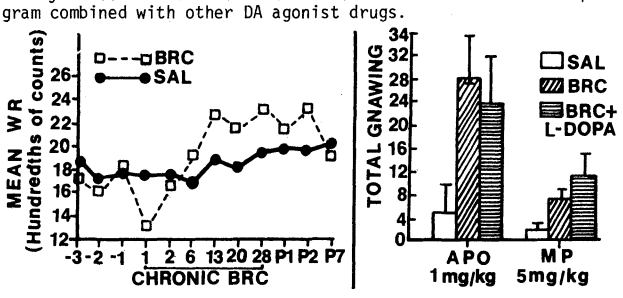
1406 BEHAVIORAL SUPERSENSITIVITY TO BROMOCRIPTINE IN MICE. Paul B. Hicks*, J. Randolph Strong*, Robert C. Smith, and Thaddeus Samorajski, Tex. Res. Inst. Mental Sciences, Houston, Tex 77030.

Bromocriptine (BRC, CB-154) has behavioral properties of a directly acting DA agonist (Brit. J. Pharm. 56: 59, 1976), although recent research also indicates effects of BRC on pre-synaptic dopamine neurons (Neurosci. Abst. 2: 500, 1977). One case study (Lancet 2: 571, 1976) reported emergence of psychosis in a patient treated with BRC + L-DOPA after discontinuation of BRC and suggested that this might involve development of supersensitivity to DA receptors with chronic BRC treatment. We investigated tolerance and supersensitivity in mice who received 30 day chronic i.p. drug administration of either: 1) BRC 30 mg/kg i.p., 2) BRC 30 mg/kg i.p. + L-DOPA ∿ 1.8 mg/kg in food, 3) L-DOPA ∿ 1.8 mg/kg in food, or 4) Saline i.p. (SAL). There was a rapid development of tolerance to the effects of bromocriptine on depressing wheel running seen on the first day of BRC administration and a reversal of drug's effects as evidenced by a significant increase in wheel running (wk) compared either 1) to the BRC mice's pre-drug baseline or to 2) same day SAL controls, both during chronic administration and after termination of BRC. After termination of chronic treatment, BRC mice showed greater knawing after apomorphine (APO) 1 mg/kg and methylphenidate (MP) 5 mg/kg than SAL controls. The combination of BRC + L-DOPA had similar effects on wheel running and knawing, with a slightly greater effect on MP induced stereotyped behavior. The behavioral supersensitivity was short lived; by day 7 post-BRC wheel running had returned to SAL control levels and in week 2 the differential effects of APO and MP on BRC mice decreased considerably. Since BRC is effective in Parkinson's disease, and may be combined with other DA agonists such as L-DOPA, our results suggest a mechanism for the emergence of potential psychiatric and dyskinetic side effects when BRC is used in a treatment program combined with other DA agonist drugs.



1407 EFFECTS OF SELECTIVE CATECHOLAMINE RECEPTOR BLOCKERS ON AMPHETAMINE AND APOMORPHINE POTENTIATED ACOUSTIC STARTLE RESPONSE IN RATS. John H. Kehne* and Charles A. Sorenson. Neuroscience Program, Amherst College, Amherst, MA 01002.

It has been suggested that catecholamine (CA)-containing neurons exert a facilitatory influence on the amplitude of the acoustic startle response (ASR) in rats, based largely on the finding that treatment with high doses of amphetamine augment startle amplitude (Davis et al., Psychopharmacol., 43:1, 1975). The individual contributions of norepinephrine (NE) and dopamine (DA)-containing neurons in mediating this potentiation of startle amplitude are unclear, although a primary role for DA neurons has been suggested by the finding that low doses of the DA agonist apomorphine cause large increases in startle for the first forty minutes after injection (Davis & Aghajanian, Psychopharmacol., 47:217,1976). In an effort to clarify the individual roles of NE and DA-containing neurons in amphetamine and apomorphine potentiation of startle amplitude, rats were pretreated with either the DA receptor blocker pimozide or the alpha-NE receptor blocker phenoxybenzamine, and then treated with either d- or l-amphetamine, or apomorphine, in doses known to cause reliable increases in startle amplitude. If DA-containing neurons are primarily responsible for startle potentiation, then pretreatment with pimozide, but not with phenoxybenzamine, should block these effects. As expected, the pimozide pretreatment did completely block the facilitating effects of amphetamine and apomorphine. Furthermore, the phenoxybenzamine pretreatment did not block the d-amphetamine effect on startle, but it did block or markedly attenuate the effects of l-amphetamine and apomorphine. One possible interpretation of these results is that the startle-potentiating effects of d-amphetamine are mediated through a different CA pathway than are the effects of l-amphetamine and apomorphine, the latter effects being mediated through DA and NE neural systems that are in series. (Supported by the Alfred P. Sloan Foundation).

1408 EFFECT OF TAIL OF CAUDATE LESIONS ON SPONTANEOUS AND AMPHETAMINE-INDUCED LOCOMOTION IN RATS. Robert D. Kerns*, Amanda M. McGee*, and Luis A. Baez. Dept. Psychol., Southern Illinois University, Carbondale, Il., 62901.

Bunney and Aghajanian (Science, 1976,192,391-393) have reported that lesions of the tail of the caudate or of the crus cerebri in rats produce an increase in the firing rate of neurons in the substantia nigra. These data are consistent with other evidence suggesting that the activity of dopaminergic neurons is influenced by feedback connections from forebrain areas to which they project. Since dopaminergic mechanisms have been implicated in the control of behavioral arousal (Creese and Iversen, Brain Research, 1975,83 419-436; Roberts et al.,Brain Research,1975,93, 441-454), it might be predicted that a release of nigral neurons from inhibitory control should result in increased behavioral arousal. Bunney and Aghajanian (1976) also reported that tail of caudate lesions blocked the inhibitory action of d-amphetamine on the firing rate of substantia nigra cells, suggesting that this effect is also mediated by striatonigral connections. Thus, it might also be expected that similar lesions should potentiate the effects of d-amphetamine on behavior. The present study was designed to investigate both of these hypotheses.

In the first experiment, 18 Long-Evans adult rats were housed in stabilimeter cages with food and water freely available. After a habituation period of 36 hours, bilateral electrolytic lesions of the tail of the caudate were performed in nine rats and sham lesions in the remaining animals. Daily locomotor activity was then measured continually for four days following surgery. Locomotor activity of the lesioned group was markedly higher than that of the shams during the four days of observation and this effect was statistically significant ($p < 0.01$). Activity (stabilimeter crossings) of the lesioned group during the first postoperative day was over seven times higher than that of the shams, and it was still double that of the sham group by the fourth day.

In the second experiment, a group of ten animals with lesions of the tail of the caudate and ten sham lesioned animals were injected with 1.0 mg/kg d-amphetamine sulfate one week after surgery. Stabilimeter activity was recorded during five hours following drug administration. The mean activity of the lesioned group was 243 counts, whereas that of the sham group was 123 counts, a statistically significant difference ($p < 0.01$). A second group of animals was tested with d-amphetamine five weeks after surgery. Although the response of the lesioned group was higher than that of the shams (lesioned mean: 445; sham mean: 283) this difference failed to reach statistical significance. These data, then are consistent with the concept of a functionally significant striataonigral feedback projection.

1409 SIALIC ACID/2-DEOXYRIBOSE AS BIOCHEMICAL MARKERS FOR REGIONAL DIFFERENCES IN BRAIN EFFECT OF ACUTE AND CHRONIC ETHANOL. W. R. Klemm and R. L. Engen*, Dept. Biol., Texas A&M Univ., College Station, Tx. 77843 and College Vet. Med., Iowa State Univ., Ames, Iowa 50010.

Sialic acid, the generic term for a family of acidic aminosugars that are incorporated in both glycoproteins and glycolipids, is a potential source for many steric specificities of neuronal and glial membranes. Moreover, the association with membranes allows sialic acid to serve as a possible marker of membrane alteration or development. Therefore, we wished to compare acute and chronic ethanol effects on sialic acid. Experiments were conducted in 50 adult, male Wistar rats, housed in groups of 5. After a 1-month acclimation period, when body weights were reduced 13% from a beginning mean level of 407 grams, experimental rats drank ad libitum a vitamin-fortified diet (Nutrament) which was adulterated with an ascending series of ethanol; ethanol intake ranged from 10-18 gm/kg/rat/day and did not vary much with concentration (5 to 15%). Controls were fed an equal total volume, made isocaloric with sucrose. Rats were sacrificed weekly for 4 weeks, and an acute challenge dose of ethanol (2 gm/kg, IP) was given 45 min prior to sacrifice of both control and ethanol-consuming rats (which were tolerant after 1 week). Some controls were challenged only with saline. Data from all groups replicated our earlier findings of regional differences in sialic acid (descending concentration: telencephalon → cerebellum → medulla). Also replicated was the observation of about a 4-fold higher level of cerebellar deoxyribose, which was measured as a necessary adjunct in the analysis procedure (autoanalyzer modification of the Warren-Delmotte methods). Saline-challenged controls that were sacrificed at 4 weeks had increases throughout the brain in both sialic acid and deoxyribose. Ethanol-challenged controls had a slight, nonsignificant tendency for less sialic acid, but a marked decrease in cerebellar deoxyribose. In contrast, such a challenge of rats on the ethanol diet caused no such decreases; significant differences in both sialic acid and deoxyribose were evident within 2 weeks, especially in the cerebellum.

If we can assume that sialic acid levels reflect the relative amount of cell membranes, and that deoxyribose reflects the number of cells, results in saline-challenged controls suggest that growth may have occurred during the 3 weeks, even though rats were fully adult. For the ethanol data, two interpretations must be considered; namely, that the chronic treatment either 1)created a tolerance which protected cells from damage by the challenge dose of ethanol, or 2) killed neurons, and then the smaller and more numerous glial cells proliferated.

1410 MUTUAL ANTAGONISM OF BEHAVIORAL EFFECTS OF TRH AND THIOBARBITURATE ON AN OPERANT TASK IN RHESUS MONKEYS. Fredric H. Klopf*, Gary W. Kraemer*, William T. McKinney (SPON: Robert E. Bowman). Dept. Psychol. Psychiat. Univ. of Wis., Madison, WI 53706.

The effects on operant response rate of 20 mg/Kg of thyrotropin releasing hormone (TRH) and 12.5 mg/Kg sodium thiamylal (surital^{R}, P.D.) administered alone or in combination were examined on a variable interval schedule for sugar water reward in 8 rhesus monkeys. The subjects were segregated into 2 groups of 4 on the basis of baseline response rate. The high response rate group had a mean rate of 13.1 responses per minute while the low rate group had a mean rate of .36 responses per minute over a 2-hour test duration. Administration of TRH or thiamylal alone decreased rate of response in the high rate group while increasing response rate in the low rate group. These effects were over 2 hours in duration for both agents. When TRH and thiamylal were given together, the response rate altering effects of either drug given alone were cancelled and response rate approximated the baseline rate. This effect occurred when subjects had high or low baseline rates and despite the fact that the effects of either of the agents given alone were in the same direction. Results suggest that both TRH and thiobarbiturate have rate dependent effects on this operant task in rhesus monkeys but that regardless of the direction of effect, the behavioral effects of thiobarbiturates and TRH are mutually antagonistic. These findings confirm and extend previous studies documenting reversal of barbiturate effects by TRH and further suggest that thiobarbiturates may antagonize some central effects of TRH. Antagonism of a central TRH receptive mechanism may be another way in which barbiturates act to alter brain function.

1411 Acute and Chronic Effects of d-Amphetamine: Behavioral and Neurochemical Specificity. Larry Kokkinidis* and Hymie Anisman* (SPON: T. Picton). Department of Psychology, Carleton University, Ottawa, Ontario, Canada.

Acute and chronic effects of d-amphetamine were evaluated in five behavioral tasks, locomotor activity, stereotypy, stimulus perseveration, startle reflex and circling. Following administration of 10 mg/kg of the drug over five successive days tolerance was observed to the perseveration, startle reflex and rotational behavior induced by the drug, whereas the intensity of locomotor activity and stereotypy either increased or remained unchanged. Treatment with alpha-methyl-tyrosine (AMPT) (250 mg/kg), antagonized the effects of d-amphetamine in each of the behavioral tasks. Treatment with Bis-(4-methyl-1-homopiperazinyl-thiocarbonyl) disulfide (FLA-63) (40 mg/kg) on the other hand, only antagonized the effects of amphetamine on stimulus perseveration, startle response and circling, whereas locomotor activity and stereotypy were unaffected by dopamine-Beta-hydroxylase inhibition. Since AMPT reduced dopamine by 40 percent and norepinephrine by 49 percent, whereas FLA-63 reduced norepinephrine by 47 percent with no effect on whole brain dopamine levels, it is likely that these behaviors are subserved by noradrenergic activity. This finding taken together with the fact that tolerance was not observed to the locomotor and stereotypic effects of the drug, whereas the perseverative, circling and startle tendencies were attenuated with chronic drug treatment, suggests that tolerance may occur exclusively to behaviors mediated by norepinephrine.

1412 EFFECTS OF ALTERATIONS IN BIOGENIC AMINE METABOLISM ON THE PROTEST-DESPAIR RESPONSE TO PEER SEPARATION IN RHESUS MONKEYS. Gary W. Kraemer*, Fredric H. Klopf*, Steven J. Suomi* and William T. McKinney. Dept. Psychiat and Psychol. Primate Lab. Univ. of Wis., Madison, WI 53706.

Separation of rhesus monkeys from their mothers may result in a protest-despair response which is qualitatively similar to that shown by some human infants following separation from their mothers. The behavioral features of this biphasic effect, characterized by an initial phase of agitation and increased vocalization (protest) followed by decreased activity, huddling and failure to attend to the external environment (despair), have been labeled as anaclitic depression in humans. On the basis of this etiological and behavioral analogy, many investigators have suggested that despair in monkeys may serve as a primate model of some forms of human depression.

Rhesus monkeys raised without mothers in a peer group may also show a protest-despair response following separation from the peer group. The peer separation paradigm differs from mother-infant separation in that the group can be repeatedly separated and reunited, each separation resulting in a qualitatively and quantitatively similar response. This suggested that the repeated peer separation paradigm could be used to evaluate the interaction of alterations in catecholamine (CA) or indolamine (5HT) metabolism with despair in primates by administration of drugs altering biogenic amine metabolism or placebo across repeated peer separations.

In a series of repeated peer separations involving 2 groups of 4 rhesus monkeys, it was found that: a) alteration of CA metabolism with doses of alpha-methyl-para-tyrosine (AMPT) as low as 25 mg/Kg/day potentiated the despair stage following separation while having no effect on group behavior preseparation; b) inhibition of dopamine beta hydroxylase (DBH) with Fusaric acid in doses as low as 5 mg/Kg resulted in less despair behavior; c) inhibition of tryptophan hydroxylase with parachlorophenylanine (PCPA) had no effect on despair behavior in doses up to 200 mg/Kg/day over a 12-day period.

Therefore, alterations of CA metabolism which may not have detectable behavioral correlates in a social situation may significantly alter the response to peer separation in rhesus monkeys. On the basis of the known effects of the enzymatic inhibitors used, the data suggest that small decrements in norepinephrine (NE) and dopamine (DA) metabolism may potentiate despair behavior while reduction of NE alone, with a possible increase in DA following inhibition of DBH, ameliorates post-separation despair. A role for NE and DA in the mediation of despair in monkeys is supported by these studies.

1413 EFFECTS OF AMINO ACID PRECURSORS ON MONOAMINERGIC SYSTEMS IN TERRITORIAL AGGRESSION. S. M. Lasley, J. B. Thurmond*, and J. W. Brown*. Neuropsychopharm. Program, Univ. of Louisville, Louisville, Ky. 40208.

The monoamine precursors L-tyrosine, L-phenylalanine, and L-tryptophan were administered by diet and measures of aggressive behavior recorded. The dietary regimens, which were designed to enhance catecholaminergic and serotonergic functioning, were found to differentially affect the territorial-induced attacks in mice. Male CF-1 mice were maintained on a semi-synthetic 12 percent casein protein diet for 2 weeks, then switched to diets modified by the addition of a 4 percent L-amino acid supplement, or 4 percent casein (control). Measures of aggressive behavior and open-field locomotor activity were obtained before and after the dietary supplements were administered. Resident mice fed supplements of L-tyrosine displayed a marked increase in the number of attacks on intruders and shorter attack latencies, but their locomotor activity was unaffected. L-phenylalanine supplements alone or in combination with L-tyrosine reduced the latency to attack and increased motility but did not affect the number of attacks. As a whole, the group of animals fed L-tryptophan showed no changes in aggression or motility.

In order to more closely control the amount of amino acid an individual animal receives, the precursors were also administered by injection. Biochemical determinations of monoamine levels and turnover have also been made to assess the effects of the diets - especially L-tryptophan's effects - and provide a firmer base for conclusions.

1414 DOPAMINE RECEPTORS IN NORMAL AND SCHIZOPHRENIC HUMAN BRAINS. Tyrone Lee and Philip Seeman. Pharmacology Department, University of Toronto, Toronto, Canada.

In order to find out whether dopamine receptors in the brains from schizophrenic (SZ) patients differ from those in normal human brains, dopamine receptors in such brains were measured using ^{3}H-apomorphine or ^{3}H-haloperidol (Seeman et al., Proc. Nat'l. Acad. Sci., 73: 4354, 1976).

Post-mortem human brains were used. Control normal tissues were obtained through the hospital pathologist from persons who had died from non-neurological causes, while the schizophrenic brain tissue samples were contributed by Dr. W.W. Tourtellotte (NINCDS/NIMH Human Neurospecimen Bank, Los Angeles). Tissues were homogenized, incubated with ^{3}H-apomorphine or ^{3}H-haloperidol and the mixture filtered through GF/B filters and washed. Specific binding of the radioligand was defined as that amount of isotope bound in buffer minus that amount bound in the presence of (+)-butaclamol.

The highest specific binding of ^{3}H-apomorphine was in the dopamine-rich regions:

Caudate nucleus	31	fmoles/mg protein
Putamen	39	"
Nucleus accumbens	30	"
Septum	35	"

Binding to other regions was not significantly different from background (less than 10 fmoles/mg).

The specific binding of ^{3}H-haloperidol (fmoles/mg) to normal and schizophrenic brain tissues was as follows:

NORMAL	CAUDATE	PUTAMEN	N. ACCUMBENS	FRONTAL CORTEX
CAL-136	35±3	45±3	37±2	23±2
CAL-246	32±7	34±7	38	25±5
CAL-154	41	48	-	19±4
CAL-173	45±8	42±6	-	27±6
SCHIZOPHRENIC				
CAL- 18	81±8**	79±6**	-	22±3
CAL-237	91±8**	67±3**	66±4*	28±4
CAL-249	84±9**	68±4**	65±5*	23±5

*P < 0.01; **P < 0.001 (compared to the averaged normals).

It is not yet clear whether the apparently increased number of receptors in the SZ brains is independent of previous drug history.

(Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada).

1415 SITE-SPECIFIC EFFECTS OF ACUTE AND CHRONIC AMPHETAMINE ADMINISTRATION ON SELF-STIMULATION RESPONDING. Nancy J. Leith and Robert J. Barrett*, (SPON: Wolf D. Dettbarn). Dept. of Pharmacol., Sch. Med., Vanderbilt Univ., Nashville, TN. 37203

Following the initial report by Leith and Barrett (Psychopharm. 46: 19, 1976) that tolerance develops to amphetamine's facilitation of MFB self-stimulation responding, Anderson et al., (Brain Res. 1977) demonstrated that the precise location of the electrode in the hypothalamic region is critical in this phenomenon. Although the drug facilitated responding in all animals, tolerance developed only with placements that were in dorsal or medial hypothalamic regions. In an attempt to delineate more precisely the brain pathways that are involved in the development of tolerance, animals were implanted in specific brain areas that support self-stimulation (locus coeruleus (LC), ventral noradrenergic bundle (VNB), periventricular noradrenergic bundle (PNB), substantia nigra (SN), or A_{10} region) and tested following both acute and chronic amphetamine administration. The drug (0.5-0.75 mg/kg d-amphetamine sulfate) facilitated responding in all animals although the magnitude of the effect varied. VNB animals showed only slight facilitation even at the higher dose whereas in LC and PNB animals the drug effects were marked. Chronic treatment, during which no testing occurred, consisted of 3 injections daily for 4 days beginning with 1 mg/kg and incrementing in 1 mg/kg steps at each injection. Forty-eight hours after the last injection, animals were again tested for facilitation with 0.5-0.75 mg/kg d-amphetamine sulfate. All animals with electrodes aimed at the SN (n=5) developed tolerance while none of the PNB (n=5) animals did so. In the cases of the LC (n=5), VNB (n=4) and A_{10} (n=9) regions, some animals developed tolerance while others did not. In addition, testing on the subsequent day with no further drug administration showed that nearly all animals (90%) exhibited a marked depression of responding and this depression was independent of whether or not tolerance had developed. Thus, even within these relatively homogenous brain regions, slight variations in electrode site make a critical difference. The necessary conclusion from these data, then, is that self-stimulation responding in the two groups of animals (tolerance and no tolerance) is mediated by different anatomical and/or biochemical substrates which chronic amphetamine effects differentially.

1416 EFFECT OF CHLORDIAZEPOXIDE (CDP), D-AMPHETAMINE (AMP), MORPHINE (MOR) AND ZIMELIDINE (Z) ON LATERAL HYPOTHALAMIC (LH) SELF-STIMULATION (SS) FOLLOWING 4-CHLORO-AMPHETAMINE (PCA) INDUCED SEROTONIN (5HT) DEPLETION. S.A. Lorens. Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Although brain stimulation reward appears to be mediated primarily by catecholaminergic systems, 5HT seems to play an important regulatory role. Thus, reduction in central 5HT concentration can lead to major changes in SS behavior depending on the site stimulated. Recently, we found that intra-midbrain 5,7-dihydroxytryptamine (5,7-DHT) injection reduced regional forebrain 5HT levels and enhanced LH SS responding. The facilitatory effects of AMP, CDP, and MOR, furthermore, were potentiated. In view of the importance of the method of 5HT depletion in determining behavioral changes (Lorens, Ann. N.Y. Acad. Sci., in press), the effects of the long term 5HT depleting agent, PCA, were examined.

All methods have been published (Psychopharmacologia, 1976, 48:217). Following stabilization of responding at threshold current intensity, rats received PCA (10.0 mg/kg; n=4) or saline (S; 1.0 ml/kg; n=4) i.p. PCA suppressed LH SS 0.5-8.0 hr. post-injection. The response outputs/10 min. of the PCA and control groups, however, did not differ over the subsequent 17 days.

Drugs were administered i.p. after a control session according to the following schedule: on post-PCA day 6 (S, 1.0 ml/kg), 7 (CDP, 2.0 mg/kg); 9 (S), 10 (AMP, 0.1 mg/kg); 12 (S), 13 (MOR, 5.0 mg/kg, s.c.); 15 (S), 16 (Z, 10.0 mg/kg). CDP enhanced SS responding 0.5 hr. after injection, while AMP was without effect. No differences between the PCA and control groups were found. MOR suppressed SS 1 hr. after injection in both groups, but the response output of the PCA group was greater 3 hr. post-injection. Z reduced (20%) SS responding 2 hrs. post-injection in the control group, but not in the PCA group.

Although PCA acutely suppresses LH SS, it does not appear to produce long term changes in SS response output. PCA *antagonized* the suppressive effects of Z, a selective 5HT uptake inhibitor (Ross and Renyi, Neuropharmacol., 1977, 16:57), and potentiated the facilitatory influence of MOR. PCA did not alter the effects of low doses of CDP and AMP on LH SS.

Distinctions between the effects of 5,7-DHT and PCA on SS behavior could be due to a different pattern and degree of regional 5HT depletion and resulting receptor supersensitivity. The results, nevertheless, are not inconsistent with the hypothesis that 5HT is involved in modulating brain stimulation reward, and in mediating the behavioral effects of chlordiazepoxide, amphetamine, morphine, and Zimelidine.

1417 CHLORPROMAZINE REVERSAL OF AMPHETAMINE STEREOTYPY IN AMYGDALOID RATS. Irwin N. Lourie* and Michael M. Krieger* (SPON: N. S. Thampi). Research Dept., Norristown State Hospital, Norristown, PA 19401.

An observational method for the analysis of d-amphetamine (AMP) effect on behavior in rats was developed which distinguishes a dose related continuum of behavior from hyperactivity through marked stereotypy. The procedure consists of analysis of videotape observations of normal and abnormal components of exploratory (E) and paired interactive (I) behavior. Bilateral radiofrequency lesions were placed in the amygdala (A) of ninety-six day old male NSH rats (n=10) resulting in extensive damage to basolateral and corticomedial structures. Controls (C) consisted of a similar group of sham operated animals. After 14 days of recovery animals were run daily in an open field until behavior stabilized (8 days). Three days of baseline and four days of drug trials were run. Drugs consisted of i.p. administration of 2, 4, and 10 mg/k AMP given 30 minutes prior to observation and 10 mg/k AMP preceded by 8 mg/k chlorpromazine (CPZ) given 15 minutes previously. (A) without drugs showed a typical response: rapid habituation of E and deficits in I with concommitant increases in noninteractive behavior. Both groups showed similar responses to AMP with differences consistent with lesion effect.

Reversal of 10 mg/k AMP with 8 mg/k CPZ produced a marked difference in response. For attending behavior (nonambulatory, motor activity) A showed 55% as the intense stereotyped pattern as compared with 10% for C. Thus A shows considerably less reversal response to CPZ suggesting that an intact amygdala is necessary for maximizing this effect.

1418 THYROTROPIN-RELEASING HORMONE (TRH) ELEVATES CEREBELLAR cGMP LEVELS. R.B. Mailman, R.A. Mueller, G.D. Frye* and G.R. Breese. Departments of Psychiatry, Pharmacology and Anesthesiology, and the Biological Sciences Research Center. University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

Numerous behavioral studies have indicated that TRH has central actions unrelated to its effects on the anterior pituitary. No parallel changes in CNS biochemistry have been reported. These studies investigated the effects of drug treatments on guanosine-3', 5'-monophosphate (cGMP) levels in the cerebellum of Sprague-Dawley rats. After drug treatment, the animals were killed with focused microwave irradiation (3.5 kw, 2.0 sec; Gerling-Moore Metabostat), the cerebellum was removed, homogenized in 0.4 M $HClO_4$ and centrifugated at 3000 g for 20 minutes. Aliquots of the supernatants were brought to pH 6.2 with TRIS base and analyzed for cGMP, cAMP or TRH levels by radioimmunoassay. Intravenous administration of TRH (10 mg/kg) increased cerebellar cGMP from c.12 to c.24 pmoles/mg protein. cAMP was not altered by TRH. The change in cGMP induced by TRH was dose-dependent up to 10 mg/kg, but administration of more than 10 mg/kg caused no further increase in cGMP. Cerebellar TRH "immunoreactivity" increased from c.800 ng/g cerebellum at 10 mg/kg of TRH to > 4800 ng/g at 30 mg/kg. Hypophysectomized rats responded identically to i.v. administration of 10 mg/kg TRH, indicating that the changes in cGMP were not due to actions on the pituitary. Similar dose dependent rises in cGMP in the cerebellum were seen after intracisternal injection of 3-100 µg TRH. All doses caused significant elevations, with 100 µg TRH causing a 70% increase in cGMP. The time course of both TRH and cGMP levels in the cerebellum was compared after a single i.v. injection of 10 mg/kg. Levels of cGMP were maximal at 2.5-7.5 minutes after administration of TRH, and then rapidly fell to control values by 30 minutes post-injection. This time course reasonably paralleled TRH-immunoreactivity in these samples. Many previous behavioral studies on TRH have measured its antagonism of the effects of ethanol or pentobarbital, drugs which also have been reported to cause pronounced falls in cerebellar cGMP levels. TRH significantly antagonized the decrease in cGMP caused by either ethanol or pentobarbital, and could completely prevent the decrease caused by low doses (c.1.5 g/kg) of ethanol. The effects of TRH on cerebellar cGMP provide definitive biochemical evidence that TRH has a central action unrelated to its endocrine actions. (Supported by USPHS Grants #AA-02334, AA-05047, MH-14277 and the Alfred P. Sloan Foundation.)

1419 TOLERANCE TO THE EFFECTS OF Δ^9 TETRAHYDROCANNABINOL IN ADAPTED AND NONADAPTED RABBITS. Parthena Martin* and Paul Consroe. Department of Pharmacology and Toxicology University of Arizona, College of Pharmacy, Tucson, Arizona 85721.

Although tolerance to the effects of marijuana or Δ^9 tetrahydrocannabinol (THC) occurs in many species including pigeons, rats, chickens, dogs, monkeys, frogs and man, Lipparini et al. (Physiol. Behav. 4:527-532, 1969) reported no attenuation of electroencephalographic (EEG) or behavioral responses in one rabbit given THC (3 mg/kg, i.v.) daily for 6 days. In the present study, 4 New Zealand White rabbits previously adapted to the test chamber, were given THC (.5 mg/kg, i.v.) daily and tested daily for 12 days. Since environmental familiarity has been shown to influence the acute effects of the cannabinoid (Pharmacol. Biochem. Behav. 3:173-177, 1975), 3 rabbits not previously adapted to the testing milieu were injected daily with the THC (in their home cages) but tested in the chamber only on the 1st and 12th day of cannabinoid administration. A vehicle control (10% Tween 81-saline) baseline was recorded for all 7 rabbits 1 day before THC administration. EEG, quantified by frequency analyses of delta (.5-4Hz), theta (4-8Hz), alpha (8-13Hz) and beta (13-40Hz) abundance, was recorded from the motor cortex and hippocampus for 20 minutes following THC administration. Concurrently, durations of standing, sprawling and activity were measured. Overall, adapted rabbits exhibited less delta and beta and showed a trend toward more theta and alpha cortical EEG content; they were also less behaviorally active than nonadapted rabbits. After the first administration of THC adapted rabbits exhibited more theta than nonadapted rabbits and this difference persisted with chronic THC administration. In adapted rabbits, no differences were found in EEG activity between THC and vehicle control at anytime. Behaviorally, the first injection of THC induced sprawling, reduced activity and eliminated standing behaviors. By the 12th day of THC administration, sprawling had disappeared and activity and standing tended to increase above that of baseline levels. In nonadapted rabbits, THC initially produced an increase over baseline in delta abundance of the cortical EEG which returned to baseline level by the 12th day of cannabinoid administration. Activity increased above baseline then tended to decrease with chronic administration of THC while standing tended to increase with chronic THC. These data show that tolerance to the effects of THC occurs in rabbits and that acute and chronic effects produced by THC are influenced by environmental factors. These data are also consistent with observations in other species that tolerance develops to the depressant properties but not to the stimulant effects of THC. (This research was supported by NIDA grant #DA 01448.)

1420 PHYSIOLOGICAL TOLERANCE TO SELF-ADMINISTERED d-AMPHETAMINE AFTER CHRONIC d-AMPHETAMINE INJECTIONS. Thomas McCown*, and Robert J. Barrett* (SPON: N. Leith). Dept. Pharm. Sch. Med., Vanderbilt U., Nashville, Tenn. 37235.

Rats were implanted with chronic intravenous cannulae and trained to bar press for intravenous, self-administered dextroamphetamine (d-AMPH). When baseline responding stabilized, the animals were removed from the test situation and subsequently injected, three times a day, for four days with increasing amounts of d-AMPH (total=78mg/kg) (Leith and Barrett, Psychopharm. 46: 19-25, 1976). Thirty hours after the last injection, the animals were tested and all animals significantly increased the amount of self-administered d-AMPH, as compared to baseline administration. Approximately three to four weeks of drug abstinence was necessary before the animals returned to a baseline administration.

Brain clearance and brain levels of d-AMPH, after an intravenous dose of d-AMPH, in either chronic d-AMPH or saline treated animals, were measured by the methyl orange assay (Axelrod, JPET 110: 315-326, 1954), and no differences were found in either index. The increased amount of d-AMPH administration cannot be attributed to metabolic tolerance.

These data indicate that drug self-administration in rats is a useful paradigm to study tolerance to the rewarding effects of d-AMPH and may be useful in understanding the mechanisms mediating the mood elevating properties of the drug, observed in humans.

1421 SEX-RELATED DIFFERENCES IN AMPHETAMINE-INDUCED ANOREXIA AND HYPERTHERMIA. Edwin Meyer, Jr.*, Andrew Werber* and Loy D. Lytle (SPON: L.N.Irwin). MIT, Cambridge, MA 02139.

Although the locomotor stimulant, anorexic, and hyperthermic effects of amphetamine have been well characterized in adult male animals, little information exists regarding possible sex-related differences in these drug actions. Recently, we have shown that female adult rats injected with 1.0 mg/kg of d-amphetamine sulfate are more active, for longer periods of time, than are comparably treated adult male animals. The sex-related differences in the stimulant actions of amphetamine correlate closely with sex-related differences in the accumulation of the drug in brain: whereas the half-life of amphetamine in the brains of adult male rats is only 40 minutes, it is approximately 70 minutes in adult female animals (Meyer, E. and Lytle, L.D *Fed. Proc. 36: 1033 (Abstr. 4012), 1977*).

We now report that amphetamine-induced changes in food intake and body temperature are also greatly protracted in adult female rats compared to adult males. In the first experiment, 100 day-old male or female albino rats were deprived of food and water for 24 hours, and then were injected with 0.75, 1.5, or 3.0 mg/kg of d-amphetamine sulfate (salt weight) or the 0.9% saline vehicle (1 ml/kg). Food and water were immediately returned to these animals, and total intake was measured hourly for up to 8 hours post-injection. All animals injected with amphetamine became anorexic in a dose-related manner following the drug treatment; however, female rats were made anorexic for longer time periods at each dose of amphetamine than were the male rats

In a second experiment, adult male or female rats were acclimated to a warm (25° C) environment for an hour, and were then injected with 1.0, 3.0, or 9.0 mg/kg of d-amphetamine sulfate or with the 0.9% saline vehicle. Food and water were removed, and rectal temperatures were measured at 30 minute intervals for up to 8 hours post-injection. All amphetamine-treated animals were made hyperthermic in a dose-related manner; however, the drug-induced hyperthermia was temporally much longer in female rats compared to males treated with the lower amphetamine doses Interestingly, no sex-related differences in amphetamine-induced hyperthermia were observed at the highest (9.0 mg/kg) dose of the drug.

The sex-related differences in the behavioral and physiological effects of amphetamine may be related to sex differences in the rates at which amphetamine is metabolized: 1) pretreating adult rats with a hepatic microsomal enzyme inhibitor (SKF-525A) blocks the metabolism of amphetamine and protracts its behavioral and physiological effects; and 2) male rats metabolize amphetamine (via para-hydroxylation) more rapidly than do female animals (Supported in part by NIMH grant MH-25075 and by an Alfred P Sloan Foundation Fellowship in Neurosciences)

1422 PSYCHOMOTOR STIMULANTS AND AGGRESSION IN MICE. K. A. Miczek, J. M. O'Donnell*, and V. S. Harris*. Dept. Psych., Carnegie-Mellon Univ., Pittsburgh, PA 15213.

Anecdotal and experimental observations suggest that amphetamines and other psychomotor stimulants increase aggressive behavior in several species, including man. We investigated the effects of d-amphetamine, methamphetamine, methylphenidate, cocaine, and L-DOPA on aggressive behavior between resident and intruder mice. Male resident mice were isolated or housed with a female, and were tested either in their homecages or in neutral cages with group-housed male intruder mice. Drugs were given to either the resident or the intruder mouse i.p. in a volume of 1 ml/100 g b.w. The test duration was 5 min after the first attack, 5 min being the maximal attack latency. All tests were videotaped and two observers recorded frequency and duration of agonistic and non-agonistic acts and postures. We found that (1) all drugs decreased the frequency of attack, sideways threat movements, and tail-rattle in a dose-dependent manner, the highest doses producing statistically reliable, strong suppression (2, 8 mg/kg d-amphetamine, methamphetamine and cocaine; 48, 96 mg/kg methylphenidate; *100, 200* mg/kg L-DOPA); (2) isolated mice were 2-4 times less sensitive to the antiaggressive effects of d-amphetamine, methamphetamine, cocaine, and L-DOPA, than mice housed with a female, whether tested in their homecages or in neutral cages; (3) 2 mg/kg d-amphetamine suppressed attack, sideways threat movements, and tail-rattle in tests 5, 15, and 30 min after injection, while non-agonistic locomotion remained unaffected; (4) doses of d-amphetamine and cocaine which suppressed attack did not alter non-agonistic locomotion, but the same doses of methamphetamine and methylphenidate increased locomotion, and L-DOPA nonspecifically decreased all agonistic and non-agonistic behaviors; (5) non-drugged resident mice attacked intruders which were given 8 mg/kg d-amphetamine or methamphetamine, or 96 mg/kg methylphenidate more frequently than those given saline; (6) administration of d-amphetamine (8 mg/kg), methamphetamine (8 mg/kg), or methylphenidate (48, 96 mg/kg) to intruder mice increased escape frequency and decreased the duration of the defensive upright posture, while increasing non-agonistic locomotion. By contrast, L-DOPA nonspecifically suppressed all intruder behaviors. We conclude that d-amphetamine, methamphetamine, methylphenidate, cocaine, and L-DOPA fail to increase any element of the species-specific pattern of attack behavior in mice, but rather decrease this behavior. The specific antiaggressive effect of psychomotor stimulant drugs could not be mimicked by L-DOPA, which had entirely nonspecific suppressive effects on all aspects of behavior. (This research was supported by USPHS Grant DA 01502).

1423 EFFECTS OF REDUCING BRAIN CATECHOLAMINES ON ACTIVITY AND ON METHADONE INDUCED CHANGES IN ACTIVITY OF DBA/2 AND C57BL/6 MICE. Lawrence D. Middaugh, Samuel K. Parrish, Jr.* and John K. Nash.* Depts. of Biochem. and of Psychiatry and Behav. Sci., Med. Univ. of South Carolina, Charleston, SC 29403.

A number of reported studies demonstrate that various inbred strains of mice have different behavioral responses to psychoactive drugs. Many of these responses are thought to be mediated via drug action on neural systems utilizing catecholamines as neurotransmitters and it has been hypothesized that strain differences in these systems might account for the different responses to drugs. The narcotic analgesics, morphine and methadone have been reported to increase turnover of dopamine in rat brain. In addition, both drugs increase activity of C57 BL/6 (C57) mice but reduce activity of DBA/2 (DBA) mice. Strain differences in catecholamine systems may count for the different behavioral response to narcotic analgesics. The purpose of the experiments reported here was to determine the effect of reducing brain catecholamine concentrations on activity of C57 and DBA mice and on the methadone inducèd changes in activity of the two strains.

α-Methyl-p-tyrosine methyl ester hydrochloride (AMPT)(150, 175, 200, 250 and 300 mg/kg) was injected I.P. (.02 ml/g body weight) 3 hr prior to a 5 minute activity test. The three highest doses produced significant and increasing reductions in activity of C57 mice but did not alter activity of DBA mice.

In the second experiment AMPT (150, 175 or 300 mg/kg) was injected 3 hr prior to killing and brains were assayed for norepinephrine (NE) and dopamine (DA). Increasing doses of AMPT reduced NE and DA to about the same degree in both strains of mice. NE was reduced by 14%, 19% and 32% for C57 mice and by 8%, 25% and 33% for DBA mice. DA was reduced by 10%, 16% and 21% for C57 mice and by 11%, 12% and 18% for DBA mice.

In the final experiment, mice were injected I.P. with AMPT (175 mg/kg) or its water vehicle three hours prior to testing and then subcutaneously with methadone hydrochloride (15 mg/kg) or its saline vehicle 5 min prior to testing. As previously reported, methadone increased activity of C57 mice and decreased activity of DBA mice. AMPT at this dose did not alter the activity of either strain. Pretreatment with AMPT however, did attenuate the methadone induced elevated activity of C57 mice but did not alter the reduced activity of DBA mice.

The results of these experiments demonstrate first, that catecholamine reduction influences the behavior of C57 mice more than that of DBA mice. Second, an adequate store of catecholamine is required for methadone induced elevated activity (observed in C57 mice) but appears to be unrelated to the depressed activity (observed in DBA mice) following methadone injections. Supported in part by Gen. Med. & Fac. Res. Approp. and by PHS Grant DA01750-01.

1424 EFFECTS OF MONOACYLCADAVERINES ON MOLLUSCAN NEURONS. Mark Miller* and Matej Stepita- Klauco. Dept. of Biobehavioral Sciences, Univ. of Conn., Storrs, Conn. 06268

The presence of acylated cadaverines -- monoacetylcadaverine and monopropionylcadaverine in the mammalian brain and blood has been recently demonstrated by high resolution mass spectrometry (Dolezalova et. al., Mass Spectrometry in Drug Metabolism, pp. 201-213; Plenum, 1977). The effects of these compounds upon identified neurons in the suboesophageal ganglia of the land snail Helix aspersa were tested in this study. The membrane potential was recorded intracellularly and the compounds were iontophoretically applied from multibarreled micropipettes onto somatic and axonal regions of studied neurons.

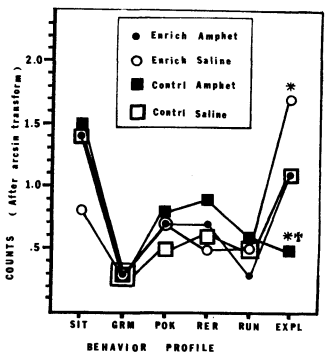
A very slowly decrementing depolarization lasting for up to one minute was produced in many cells. This depolarization was concomitant with the reduction of the membrane resistance. In addition, both the number and amplitude of spontaneously occurring postsynaptic potentials were very often increased for up to several minutes following the application into the neuropil.

Interactions of these compounds with some neuropharmacological agents were also investigated. Priming iontophoresis of a phenothiazine derivative fluphenazine was found to potentiate the depolarizing effect of monopropionylcadaverine. This effect underwent a rapid desensitization and could rarely be elicited in more than two consecutive repetitions. Longer intervals between administrations increased the probability of obtaining responses.

The effects of monoacylated cadaverines could be obtained by releasing about 1 pmole of the tested substance from a pipette containing it in a 500 mM solution. In contrast, iontophoresis of cadaverine alone was unable to produce any of the aforementioned responses. It is suggested that the acylation of one amino group of cadaverine could render a compound which is capable of exerting neuromodulatory effects.
(Supported by PHS grants NS11716 and NS12482)

1425 ENVIRONMENTAL ENRICHMENT AND ATTENTIONAL DEFICITS DURING AMPHETAMINE WITHDRAWAL. K. Nau*, H. Schreiber, R. Bell, J. Newbery* and J. Elias*. Dept. Psychol., Texas Tech U., P.O. Box 4100, Lubbock, TX 79409.

Well-known is that environmental enrichment may influence brain morphology and physiology in addition to its behavioral effects. Recent studies in our laboratory have shown that prolonged amphetamine or apomorphine administration (Schreiber, et al., Pharmac Biochem Behav:5, 687, 1976; Schreiber, et al., in prep.) may diminish response to a novel object independently of other behavioral effects during drug withdrawal. The present study showed that environmental enrichment ameliorated the diminished reaction to novelty seen after amphetamine administration. Male Fischer rats were placed in an enriched environment every other day from 26 to 100 days of age (D/age) or were left in their individual home cages. At 150±5 D/age, rats were randomly assigned to a d-amphetamine or saline injection group. Amphetamine was injected twice daily for 4 days at 2.5 mg/kg, s.c., followed by twice daily injections of 5.0 mg/kg, s.c. for 4 days. On Day 9, all Ss received saline injections. Home cage observations showed no differences among groups in general activity level (Figure not shown). However, when a 5.5 cm high X 3.5 cm diameter cylinder was placed in the home cage, Ss which had received amphetamine showed significantly less attention to the cylinder than did Ss which had received saline. As shown in the figure below, enriched, saline-injected Ss (N=3) showed greater exploration than control, saline-injected Ss (N=3). Moreover, enriched, amphetamine-injected Ss (N=4) showed more exploration of the object than control, amphetamine-injected Ss (N=3). No significant differences were detected among the other responses observed (sitting, grooming, nose-poking, rearing and running).



*indicates signif. diff. $p < .05$ from other drug group (amphet vs sal)

†indicates signif. diff. (enrich vs control)

1426 EFFECT OF THREE BENZHYDRYL PIPERAZINES ON CONDITIONED AVOIDANCE BEHAVIOR IN THE RAT. Gary D. Novack, Larry G. Stark, and A. James Hance*. Dept. Pharmacol., Univ. Calif. Davis Sch. Med., Davis. CA. 95616

The effect of three benzhydryl piperazines, SC-13504 (an anticonvulsant), hydroxyzine (HDX, an anxiolytic) and chlorcyclizine (CCZ, an antihistaminic) on conditioned avoidance behavior in the rat has been observed. CCZ and HDX (10 to 80 mg/kg, i.p.) decreased both pre-tone avoidances (secondary conditioned responses) and post-tone avoidances (conditioned avoidance responses) at doses one-fourth to one-half of those which abolished escapes. High doses of HDX and CCZ elicited tremors and seizures. SC-13504 (20 to 160 mg/kg, p.o.) did not significantly decrease pre-tone or post-tone avoidances, nor elicit any gross behavioral effects.

The failure of SC-13504 to significantly decrease conditioned avoidance responses is consistent with previous reports of its minimal effects on behavior. Additionally, SC-13504 has been reported to be a more effective anticonvulsant than HDX or CCZ (Novack and Stark, Proc. West. Pharmacol. Soc., in press). Based on its minimal behavioral effects, SC-13504 appears to be the most selective anticonvulsant of the three benzhydryl piperazines tested here.

1427 EFFECTS OF THE TRICYCLIC ANTIDEPRESSANTS DESIPRAMINE AND DOXEPIN ON ANTICHOLINERGIC AND CNS ACTIVITY IN NON-DEPRESSED VOLUNTEERS. George R. Peterson*, Russell M. Hostetler*, Barry Blackwell*, Ronald Kuzma*, and Allen B. Adolphe. Depts. of Pharmacology & Psychiatry, Sch. Med., Wright State U., Dayton, OH 45435, and Epidemiology and Biostatistics, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Using methodology developed by this group (Blackwell et al, Psychopharmacologia 25:205, 1972), dose response relationships for anticholinergic activity, quantified by measurements of salivary flow, and mood, quantified by Clyde Mood Scale factor scores for subjective CNS states, were demonstrated for tricyclic antidepressant drugs (TADs). In the most recent study, the widely used TADs desipramine (Norpramin) and doxepin (Sinequan) were compared. The trial was of double-blind crossover design involving 10 non-depressed female volunteers, with each subject randomly assigned to each of 5 treatments (5 x 5 Latin square). Placebo and doses of 50 and 100 mg for both drugs were used. Salivary flow, an index of anticholinergic activity, was significantly depressed by active drug treatment. Doxepin, however, produced significantly greater reduction in salivary flow than desipramine at the higher dose ($p<0.05$). The Clyde Mood Scale factor scores for the subjective states of "friendly", "aggressive", "dizzy", "clear thinking", "sleepy" and "unhappy" were also tabulated. Significant differences were observed in 4 of the 6 scores: "sleepy", "friendly", "aggressive", and "clear thinking" ($p<0.05$). In 1 of the 4 ("sleepy"), the impairment produced by doxepin was significantly greater than desipramine's at both doses ($p<0.05$). The quantified Clyde Mood Scale scores are in good agreement with the subjective complaints volunteered by the study participants. In addition, the subjects consistently made more complaints and rated these experiences more severe after taking doxepin than after desipramine at both doses. These observations, and related clinical studies by this group involving other TADs (imipramine and amitriptyline), are in good agreement with recently reported in vitro and animal studies relating anticholinergic and CNS effects to one another and to TAD structure. These studies have succeeded in demonstrating concurrent anticholinergic and sedative effects although dose response relationships for the separate actions have differed.

1428 MAO INHIBITION AND THE EFFECTS OF CENTRALLY ADMINISTERED LSD, BROM-LSD (BOL), SEROTONIN (5-HT) AND 5-METHOXYTRYPTAMINE (5-MT) ON THE CONDITIONED AVOIDANCE RESPONSE (CAR) IN RATS. Walter C. Prozialeck and W.H. Vogel, Department of Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107

In order to clarify the behavioral effects of 5-HT, 5-MT, LSD and BOL, we examined the effects of these compounds on the CAR in normal and iproniazid pretreated rats. In addition, we determined the brain levels of centrally administered LSD and 5-MT at a time after injection that coincided with the peak behavioral activity of these compounds.

Drugs, dissolved in artificial CSF (10 μl), were injected into the right lateral ventricle via chronically implanted canulae. Behavioral tests were conducted in a standard shuttlebox apparatus (Cohen et al., Psychopharm. 12, 1214, (1972).

The intraventricular administration of artificial CSF, BOL (5μg), 5-HT (creatinine sulfate complex, 8.5 μg) and 5-MT (hydrochloride, 5-μg) had no significant effect on CAR performance. LSD (5μg) produced a marked disruption of the CAR lasting for $\sim$15 min. Iproniazid pretreatment alone (50 mg/kg 15 hrs prior to testing) had no effect. Following iproniazid pretreatment, 5-MT caused a marked disruption of the CAR with an increase in escape time and a decrease in the number of premature responses. 5-HT had a less pronounced but still significant effect on the CAR and increased reaction time. BOL had no effect. Surprisingly, the behavioral response to LSD was abolished by iproniazid pretreatment. This decrease in the response to LSD was also observed when the compound was given I.P. (300 μg/kg).

Ten minutes after intraventricular administration, whole brain levels of 5-MT were markedly higher in the iproniazed treated animals (1.5$\pm$.1μg) than in the non-treated animals (.06$\pm$.01 μg). Levels of LSD were not affected by MAO inhibition (.19$\pm$.10 μg vs. .15$\pm$.02 μg for the controls).

In summary, our data show a marked reduction in the behavioral effects of LSD and a marked increase in the effects of 5-MT and 5-HT following MAO inhibition. Levels of injected 5-MT are significantly elevated after MAO inhibition which probably results in the increased behavioral effects, whereas levels of LSD are not affected while the behavioral response is abolished. These findings, along with the fact that each drug has a slightly different effect on shuttle-box performance, suggests that LSD, 5-HT, and 5-MT may each exert their behavioral effects via different mechanisms within the CNS.

1429 AUGMENTED BEHAVIORAL RESPONSIVENESS TO CENTRALLY ADMINISTERED CATECHOLAMINE AGONISTS FOLLOWING LONG-TERM AMPHETAMINE TREATMENT. George V. Rebec and David S. Segal. Dept. Psychiat., Sch. Med., UCSD, La Jolla, CA 92093.

We have previously reported that long-term administration of d-amphetamine produces a progressive augmentation of amphetamine-induced psychomotor stimulation in rats. To determine the involvement of central mechanisms in the augmented behavioral response and to rule out changes in peripheral dispositional factors, we monitored the behavioral response to a variety of direct and indirect acting catecholamine agonists infused into the lateral ventricle of freely moving rats pretreated with subcutaneous injections of saline or 5.0 mg/kg d-amphetamine for 4 consecutive days. Infusion cannulae were stereotaxically implanted in the lateral ventricle at least one week prior to the initiation of the experiments. Approximately 24 hours after the last systemic injection of saline or d-amphetamine, the animals were connected to an intraventricular infusion apparatus and allowed one hour of habituation. Various doses of the test drugs were infused at a rate of 20 μl/hr for up to 3 hours. In amphetamine pretreated rats, an intraventricular infusion of d-amphetamine elicited a 50-100 per cent greater increase in locomotor activity than in saline pretreated controls. The behavioral response to central administration of direct acting catecholamine agonists (apomorphine, dopamine, norepinephrine) was also enhanced in rats receiving long-term amphetamine treatment. These results suggest that a facilitation of catecholaminergic transmission is involved in the chronic amphetamine-induced behavioral augmentation.

1430 The effect of Tryptophan Depletion on Brain 5-HT and the Response to Ethanol Administration. R.F. Ritzmann and B. Tabakoff Dept. of Physiology, U. of Ill. Medical Center, Chicago, IL.

Interactions of ethanol with serotonin (5-HT) systems in brain have been the subject of much recent research. In the present study 5-HT levels were modulated by omitting tryptophan (TRY) from the diets of mice for 4 days. Control mice received a similar diet with TRY added. Mice were pair fed to obtain equal control for weight loss. Two additional group fed the TRY deficient diet were given either daily injection of kynurenine (KYN) (75 mg/kg) or a single injection of TRY (75 mg/kg) 3 hours before testing. The result of these treatments on brain TRY and 5-HT was a 60 % reduction in TRY and a 50 % reduction in 5-HT in the mice receiving the low TRY diet as compared with normal pair fed animals. The single injection of TRY restored both TRY and 5-HT, while KYN had no effect on these levels. In the first series of experiments, the ability to metabolize ethanol was determined. Blood samples were taken at various times after the ethanol injection (3 gm/kg), and the amount of ethanol was determined by gas chromatography. Additional mice were decapitated and brain ethanol levels were measured. These studies indicate that the low TRY treatment results in a slower rate of ethanol metabolism. The daily treatment with KYN reversed this effect while the single injection of TRY was ineffective. Similarly treated groups of mice were used to determine the effect of the dietary treatments on sleep time and ethanol induced hypothermia. The results of these studies indicate that the low TRY fed mice were more sensitive to ethanol on both variables than normal TRY fed controls. The single injection of TRY reversed this increased sensitivity while KYN only slightly reduced this effect. These results indicate the effects of acute ethanol on sleep time and hypothermia may be mediated by 5-HT. The mechanism of this response is currently under investigation in our laboratory.

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1431 EFFECTS OF PROPRANOLOL AND PHENTOLAMINE ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT. Helen Savaki*, Massako Kadekaro* and Louis Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, Md. 20014.

Propranolol blocks the action of sympathomimetic amines on β-adrenergic receptors whereas phentolamine blocks many responses that involve α-adrenergic receptors. The [^{14}C]deoxyglucose method measures the rates of local cerebral glucose utilization, and it has proved useful for mapping functionally related areas of the brain in various physiological and pathological states. This method has been applied to study the effects of propranolol and phentolamine administration in the rat. Normal conscious rats were infused intravenously with 0.25, 0.5, or 1.0 mg/Kg/min of propranolol or 0.25, 0.5, or 0.83 mg/Kg/min of phentolamine. Forty min after the administration of the drug local cerebral glucose utilization was measured during a 45 min period. Propranolol caused dose-dependent reductions in cerebral glucose utilization in several regions of brain, most prominently in the structures of the auditory pathway. Phentolamine also caused local reductions of cerebral glucose utilization in a number of cerebral structures but, in contrast to propranolol, caused increases in glucose consumption in the auditory system. These results suggest that adrenergic synapses may play an important role in the function of the auditory pathway of the rat.

1432 THE BEHAVIORAL EFFECTS OF CHRONIC ADMINISTRATION OF D-AMPHETAMINE AND APOMORPHINE AND THE DEVELOPMENT OF CONDITIONED STEREOTYPY. STANLEY R. SCHIFF* AND WAGNER H. BRIDGER. ALBERT EINSTEIN COLLEGE OF MEDICINE, DEPARTMENT OF NEUROSCIENCE, BRONX, NEW YORK 10461.

Male, hooded rats were randomly assigned to drug groups (1) saline control, (2) amphetamine hydrochloride 1.0 mg/kg, (3) amphetamine hydrochloride 6.0 mg/kg, (4) apomorphine 2.5 mg/kg, (5) apomorphine 5.0 mg/kg , and (6) pseudo-conditioning control-saline as drug treatment with amphetamine 1.0 mg/kg following the experimental session. The experimental session was conducted as nearly as possible at the same time each day, and consisted of (1) weigh animal, (2) expose to tone (CS) for 1 min. in injection cage, (3) inject animal with drug (UCS) and immediately place in observation aquarium, (4) score animal's behavior (UCR) for 2 min. intervals at 7 min., 16 min., and 25 min. post injection, (5) return animal to home cage. The behaviors (1) licking, (2) chewing, (3) head bobbing, (4) sniffing, (5) crossing(forepaws crossing into separate quadrants of the observational cage - frequency), (6) rearing (both forepaws leave floor - time and frequency), (7)forepaw pacing, and (8) grooming , were rated blind using microswitches and automatic counters through a two-way mirror from a sound-proof room. Day 1 was a baseline saline control day for all animals, days 2-11 were drug treatment days, while days 12-16 were again saline days. Significant conditioning was found for the head bobbing component of stereotypy for the amphetamine groups only, although not for locomotor (5 and 6) or other components. In addition, head bobbing showed potentiation over trials (Amp 1 mg/kg) , while licking and chewing showed tolerance over trials (Apo 5 mg/kg).

1433 NEUROLEPTIC ANTAGONISM OF BEHAVIORAL EFFECTS INDUCED BY 5-METHOXY N,N-DIMETHYLTRYPTAMINE IN MONKEYS. R.F. Schlemmer, Jr., N. Narasimhachari, & J.M. Davis. Ill. State Psychiatric Inst., Chicago, IL 60612.

5-Methoxy N,N-dimethyltryptamine (5-MeODMT) has been reported to be hallucinogenic in humans and has been considered as a possible endogenous psychotogen. We have previously reported that administration of 5-MeODMT to monkeys induces profound alterations in social and solitary behavior (Comm. Psychopharmacol. 1:105, 1977). Acute & chronic administration of 5-MeODMT, 0.25 mg/kg, to selected members of primate social colonies induces dog-like wet shakes, involuntary limb jerks, hypervigilance, & occasional stereotypies. The same dose decreases social groom & increases submissive gestures in most treated animals. Because of the similarities of several of the behavioral changes induced by 5-MeODMT to changes induced by d-amphetamine in this species, the effect of a neuroleptic agent on 5-MeODMT-induced behavioral changes was studied. Following the assessment of behavioral effects induced by i.m. administration of 0.25 mg/kg 5-MeODMT to selected members of a Stumptail macaque social colony, the experiment began with a 5 day baseline observation period. Haloperidol (HAL), 0.05 mg/kg, was then administered i.m. twice daily for 7 consecutive days. During the following wk., HAL and 5-MeODMT, 0.25 mg/kg, were administered concomitantly. Behavioral observation by an experienced, "blind" observer occurred for 1 hr. daily for 5 days each wk. The last HAL dose was given 2½ hrs. prior to observation. On designated days, vehicle or 5-MeODMT was administered i.m. to the treatment group monkeys 5 mins. prior to observation. HAL antagonized 5-MeODMT-induced hypervigilance while reducing the number of wet shakes induced by the hallucinogen. HAL also antagonized 5-MeODMT-induced increases in submissive gestures. A tongue protrusion dyskinesia induced by 5-MeODMT in 1 monkey was antagonized by HAL. However, the HAL + 5-MeODMT treated monkeys did not appear normal, frequently assuming abnormal postures & staring into space. 5-MeODMT-induced limb jerks were not antagonized by HAL in all treated monkeys. Social grooming scores, reduced by 5-MeODMT alone, were not returned to baseline levels by HAL. Pilot studies in individual monkeys with the neuroleptic methiothepin (MET) have yielded more favorable results. MET, 0.1 mg/kg, administered i.m. 2 hrs. prior to observation antagonized 5-MeODMT-induced wet shakes, limb jerks, & hypervigilance. These monkeys did not assume abnormal postures. Studies concerning the effect of MET on 5-MeODMT-induced behavior in primate social colonies are presently underway in our laboratory.

1434 EFFECTS OF INFANTILE STRESS ON ADULT AMPHETAMINE-INDUCED LOCOMOTOR ACTIVITY. H. Schreiber, K. Nau*, J. Elias*, J. Eatwell*, P. Reid* and R. Bell. Dept. of Psychol., Texas Tech Univ., P.O. Box 4100, Lubbock, TX 79409.

This study determined whether mild stress in infancy influences adult responsiveness to d-amphetamine. Fischer rat pups were removed from the nest for 3 min daily for days 2 through 7 after parturition in a typical early handling (EH) procedure (See Denenberg, 1967). Control (C) pups were left undisturbed except to cull litters (N=8). At 100±5 and 350±5 days of age (D/age), male Ss were tested once in a cross maze and once in an open field. At 350±5 D/age, Ss were tested once in a passive avoidance task. At 365±5 D/age, Ss were tested in a shock avoidance, T-maze discrimination learning and reversal task. These non-drug data are to be reported elsewhere (Elias, et al., in preparation). At 450±30 D/age (at least 2 wks after completion of the T-maze task), EH Ss were randomly assigned to a d-amphetamine (2.5 mg/kg, 2.5 mg/cc, s.c.) group (EH/Amph, N=5) or a saline group (EH/Sal, N=4). Also randomly assigned were non-handled subjects (C/Amph, N=7; C/Sal, N=7). Ss were injected once daily 30±5 min prior to observation for 2 min in a Y-maze over 8 days. As expected, C/Amph Ss showed significantly more locomotor activity than C/Sal Ss on the initial injection. Also as expected, C/Amph Ss showed progressively more stereotypy and progressively less locomotor activity across the 8 days of injection. EH/Amph Ss showed significantly less locomotor activity than C/Amph Ss on the initial injection; however the locomotor activity level of EH/Amph Ss failed to decline across injections despite an increase in stereotypy. Because EH/Amph Ss did not show elevated locomotor activity levels when later tested in the Y-maze in the absence of amphetamine injection, drug-conditioning of activity levels (See Pickens and Dougherty, 1971) could not explain the failure of this group's activity level to decline. No differences were found in urinations/defecations or in number of Y-maze arms entered. These results, which essentially replicated previous work in our laboratory (Schreiber, et al., in preparation), indicated that early infantile stress could influence the level of d-amphetamine-induced locomotor activity in adult animals. However, in contrast to previous work in our laboratory, this finding indicated that early handling's influence on amphetamine-induced locomotor activity was not tied to the level of amphetamine-induced stereotypy through response competition or interference.

1435 EFFECT OF SELECTIVE REDUCTION OF BRAIN CATECHOLAMINES ON ACTIVITY AND AVOIDANCE PERFORMANCE IN THE DEVELOPING RAT PUP. Bennett A. Shaywitz, Todd C. Grey*, and Judith W. Gordan*. Dept Ped. and Neuro., Yale Un Sch. Med., New Haven, CT. 06510.

Abundant evidence from several lines of investigation suggests that the phenomenon of behavioral arousal observed in the developing rat during the first month of postnatal life is related to the maturation of catecholaminergic (CA) mechanisms. We have recently reported that selective depletion of brain dopamine (DA) in the neonatal rat pup at 5 days of age results in increased activity between 2 and 3 weeks of life and significantly impaired performance in active avoidance tasks at 20 and 26 days of age compared to litter mate controls. In order to determine whether these effects are influenced by DA or noradrenergic (NE) pathways we have extended our observations to include rat pups depleted of both CA as well as to animals depleted of NE selectively. Depletion of CA was accomplished utilizing intracisternal administration of 6-hydroxydopamine hydrobromide (6-OHDA). Activity in a normal developing rat pups in all control groups was comparable and followed the pattern described previously by us, averaging 23.0 ± 2.78% at 8 days and 35.4 ± 3.03% at 12 days of age. By 15 days activity has increased to 44.3 ± 3.72% and declined at 19 days to 40.6 ± 3.17%. Rat pups selectively depleted of NE exhibited a similar developmental profile and did not differ from controls except on day 8 when they were significantly less active, (averaging 15.9 ± 3.02%, P<0.025). Rat pups depleted of both CA were also a less active on day 8 as well as day 12 of age but by 19 days of age activity had increased significantly to 54.4 ± 5.24% (P<0.025) and remained increased at 23 days as well. Rat pups depleted of DA selectively were significantly more active than those depleted of both CA at all ages tested. Performance in both T-maze and shuttle box did not differ from controls in NE depleted animals but was significantly impaired in rat pups depleted of DA or depleted of both CA (P<0.001). Whole brain NE was reduced to 62.4% of controls in NE depleted rats. This reduction was evident in hypothalamus (28.4% controls) and pons-medulla (81.0% controls). Whole brain DA and NE averaged 60.6% and 42.6% respectively in animals depleted of both CA. In these animals, NE was reduced in hypothalamus (19.8% controls) and pons-medulla (38 6% controls) while DA was reduced in striatum (21.2% controls). Whole brain DA averaged 35.5% in DA depleted pups with depleting of 98% in striatum, 28.6% in hypothalamus at 87.3% in olfactory tubercles. Our results support the notion that the phenomenon of behavioral arousal observed in the first month of postnatal life is mediated via dopaminergic mechanisms.

1436 THE EFFECTS OF SELECTED CNS STIMULANTS ON JUVENILE PRIMATE SOCIAL & SOLITARY BEHAVIOR. F.K. Siemsen*, R.F. Schlemmer, Jr., R.C. Casper*, D.L. Garver, & J.M. Davis. Ill. State Psychiatric Inst. & U. of Ill. Med. Ctr., Chicago, IL 60612.

Chronic administration of d-amphetamine (d-A) to juvenile monkeys induces several behavioral changes unlike those seen with similar administration to adult monkeys of the same species (Psychopharmacol. Comm. 2:49, 1976). On the basis of this finding, the behavioral effects of other selected CNS stimulants were examined in members of a juvenile primate social colony. Stimulants selected for this study included d-A, imipramine (Imip), pemoline (Pem) & caffeine (Caff). d-A, Imip, & Pem are all effective in treating hyperkinetic children, while Caff lacks this property. 3 members of a stable, peer-raised, juvenile Stumptail macaque social colony of 6 monkeys were selected to receive treatment during the expts. Each expt. began with a baseline observation period (base) of no less than 3 weeks. Following each base, the expts. consisted of n.g. administration of d-A 0.5 mg/kg for 4 wks., Imip 3.33 mg/kg for 3 wks., Pem 2.5 mg/kg for 5 wks., or Caff 10 mg/kg for 4 wks. Social & solitary behavior of each of the 6 monkeys was observed & recorded by an experienced, "blind" observer. d-A, Imip, & Pem all significantly decreased play activity from base levels, while all 3 significantly increased social grooming from base. d-A, Imip, & Pem all decreased vigilance scores from base levels also. Only d-A & Pem induced stereotyped behavior & significantly increased self grooming scores. d-A & Imip significantly increased huddling with eyes open. On the other hand, Caff failed to alter solitary play activity, vigilance, & self grooming from base. Caff reduced social groom from base. Similar behavioral changes induced in juvenile monkeys by the 3 antihyperkinetic agents, but not by Caff, suggests that drug-induced reduction of play activity & vigilance with increased social groom may be indicative of possible antihyperkinetic properties of drugs. To test a new drug in this paradigm, nomifensine (Nom) was administered n.g. to the same 3 juvenile monkeys. Following a 4 wk. base, Nom was administered at doses of 0.05, 0.075, 0.1, 0.15, 0.2, & 0.3 mg/kg. Each dose was given for 1 wk. Observation procedures were identical to those used previously. Nom at 0.2 mg/kg, significantly elevated social & self grooming from base. The 2 lower doses of Nom significantly elevated solitary play. However, solitary play scores showed a dose-dependent decrease from 0.05 to 0.2 mg/kg of Nom. In addition, no social play or invitations to play were observed in Nom-treated monkeys above the 0.1 mg/kg dose. It appears that Nom induces some behavioral changes in juvenile monkeys similar to d-A, Imip, & Pem. These results suggest that Nom may prove effective in the treatment of hyperkinetic children.

1437 DISCRIMINATIVE EFFECTS OF COCAINE IN THE RHESUS MONKEY. William Slikker, Jr.* and Keith F. Killam, Jr.* (SPON: Larry G. Stark). Dept. of Pharm., Sch. Med., UCD, Davis, CA 95616.

Rhesus monkeys were trained to discriminate various unit doses of cocaine from vehicle in a previously described threshold determination paradigm (The Pharmacologist 18:197, 1976). After establishing a stable intravenous cocaine threshold dose, various anorectic agents and vehicle were administered on test and control days interposed between threshold determination days.

THRESHOLD DETERMINATION DAY

P	S	S	X	X	S	S	2X								
SA							2X	2X	2X	2X	2X	S	S	S	S

CONTROL DAY

P	S	S													
SA				S					S						
INJ			V		V		V		V						

TEST DAY

P	S	S													
SA											S	S	S	S	S	S	S	S
INJ			V		Y		2Y		3Y									

(P=Prime by remote infusion; SA=Self-infusion by animal; INJ=IM injection; V=Vehicle; S=Saline; X=Drug x; Y=Drug y)

The smallest intramuscular (IM) dose to evoke 15 minutes of consistent responding was determined for each drug and ranged from 0.13-2.93 μmol/kg IM (0.025-1.25 mg/kg IM): d-methamphetamine<d-amphetamine<mazindol<cocaine<diethylpropion<phenmetrazine<phentermine<phendimetrazine<ephedrine. The duration and rate of responding increased in a dose-related manner for each agent. However, an inverted U dose response was observed in that the largest dose of each drug (20-40 X threshold) diminished responding in the middle of the session. In contrast, 3 structurally related agents, clortermine (0.1-2.0 mg/kg IM), chlorphentermine (0.1-3.0 mg/kg IM) and fenfluramine (1.0-4.0 mg/kg IM) produced less responding than did vehicle.

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1438 EFFECTS OF β-CARBOLINES AND SEROTONERGIC DRUGS ON AUDIOGENIC SEIZURES IN DBA/2J MICE. David L. Sparks* and Neil S. Buckholtz. Dept. Biochem. and Dept. Psychiatry. Behav. Sci., Med. Univ. S.C. Charleston, SC 29403.

McIsaac et al. (J. Neurochem. 19, 1203, 1972) reported that 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC)(100 mg/kg) increased brain concentration of serotonin approximately two-fold, and we (Buckholtz, Pharmac. Biochem. Behav. 3, 65, 1975) have shown that 6-MeO-THBC (100 mg/kg) effectively blocks audiogenic seizures (AGS) in 21 day-old DBA/2J mice 2 hr after injection. The present series of studies determined time- and dose-response effects for this blockade of AGS and compared 6-MeO-THBC with drugs sharing some common effects.

At a 100 mg/kg dose, 6-MeO-THBC was effective at blocking AGS between 10 min and 12 hr after injection, with maximal inhibition at 1 hr. The dose-response effect at 1 hr showed that 6-MeO-THBC was effective at blocking AGS at doses between 25 and 100 mg/kg with a maximal effect at 100 mg/kg. Other β-carbolines were also tested at 1 hr at a 50 mg/kg dose, and their effectiveness at blocking AGS relative to 6-MeO-THBC (50 mg/kg) was as follows: 6-MeO-THBC = THBC > norharman > 6-MeO-tetrahydroharman > tetrahydroharman. Pretreatment with the proposed serotonergic receptor blockers cyproheptadine (7.5 mg/kg), methergoline (3 mg/kg), and methysergide (5 mg/kg), the 5-hydroxytryptophan (5-HTP) decarboxylase inhibitor RO4-4602 (25 mg/kg), or the monoamine depletor reserpine, had no effect on the action of 6-MeO-THBC.

Reference drugs with known actions were also tested. These included the following: the monoamine oxidase (MAO) inhibitors pargyline (50 mg/kg) which effectively blocked AGS, and clorgyline (5 mg/kg) and deprenyl (5 mg/kg) which had little or no effect; the uptake blockers chlorimipramine (25 mg/kg) which blocked AGS and fluoxetine (Lilly 110140, 10 mg/kg) which had no effect; the serotonergic receptor agonists quipazine (10 and 20 mg/kg) which blocked only the death component and 5-methoxy-N,N-dimethyltryptamine (5 mg/kg) which had no effect; the serotonin precursor 5-HTP (50 and 100 mg/kg) blocked AGS.

From these data we cannot isolate any one particular action of 6-MeO-THBC (e.g. increased serotonin concentration, uptake inhibition, MAO inhibition, receptor agonist) which produces the blockade of AGS. It may be that some or all of these acting together are involved. However, the fact that none of the receptor blockers affected the response to 6-MeO-THBC may indicate that 6-MeO-THBC acts on a population of serotonergic receptors not affected by these blockers or that it is involved with another neurotransmitter which cannot be manipulated by serotonergic drugs. (Supported in part by P.H.S. grant MH-26712).

1439 COMPARATIVE EFFECTS OF HALLUCINOGENIC DRUGS ON ROTATIONAL BEHAVIOR FOLLOWING UNILATERAL 6-HYDROXYDOPAMINE LESIONS. Michael E. Trulson, Arlene D. Stark* and Barry L. Jacobs. Dept. Psychol., Princeton Univ., Princeton, NJ 08540

The dopaminergic actions of various hallucinogenic drugs was assessed by examining their effects on turning behavior in rats with unilateral 6-hydroxydopamine lesions of the nigro-striatal pathway. LSD (0.1 and 0.2 mg/kg) produced strong contralateral turning, indicating that it is a potent dopamine receptor agonist, while BOL (5 mg/kg), a non-hallucinogenic congener of LSD, was found to be a weak dopamine receptor agonist. STP (2 and 5 mg/kg) and mescaline (50 and 100 mg/kg) produced significant ipsilateral turning, indicating that these compounds have a moderate dopamine-releasing action. DMT (10 and 20 mg/kg) and 5-M-DMT (0.75 and 1.25 mg/kg) produced weak ipsilateral turning, which, however, was not significantly different from that produced by the non-hallucinogenic compounds tryptamine (40 mg/kg) or scopolamine (0.25 mg/kg). Psilocin (1 to 20 mg/kg) produced no significant turning in either direction.

Previous studies revealed that a common property of many hallucinogenic drugs is their ability to inactivate the brain serotonin system by depressing raphe neuronal activity. Furthermore, the relative potency of hallucinogens in depressing the activity of raphe neurons generally parallels their potency on psychological and perceptual measures in humans (LSD > STP > Psilocin > DMT > Mescaline). Together, these studies suggested that the efficacy of a drug in producing an hallucinogenic experience may be a function of the drug's effect on raphe neurons. However, the present data demonstrate that the more potent hallucinogens also activate dopamine receptors, either directly or via the release of stored dopamine. Thus, the present data, in conjunction with previous studies, indicate that while inactivation of the brain serotonin system may be a necessary and sufficient condition for hallucinogenesis, the ability to activate dopamine receptors may be an important additional factor which determines the potency of hallucinogens.

1440 PHENELZINE POTENTIATION OF 5-METHOXY N,N-DIMETHYLTRYPTAMINE-INDUCED BEHAVIORAL CHANGES IN PRIMATE SOCIAL COLONIES. C.B. Tyler, R.F. Schlemmer, Jr., N. Narasimhachari, G.N. Pandey, & J.M. Davis. Ill. State Psychiatric Inst., Chicago, IL 60612.

5-Methoxy N,N-dimethyltryptamine (5-MeODMT), a hallucinogen, has been reported to induce abnormal behavior as well as altering normal affiliative behavior in monkeys. A previous report indicates that 5-MeODMT-induced hyperactivity in rats is potentiated by monoamine oxidase (MAO) inhibition. To study the effect of this combination in monkeys, a stable social colony of 5 adult Stumptail macaques was divided into 2 treatment groups of 2 & 3 monkeys respectively. The experiment started with a 1 week behavioral baseline when vehicle (Veh) was injected in all 5 animals prior to observation. At designated times during the study, each group received an i.m. injection of 5-MeODMT at doses of 50, 200, & 250 µg/kg for 5 consecutive days each. The group not treated with 5-MeODMT at that time received Veh i.m. Also during the study, each of the 2 groups received n.g. treatment with the MAO inhibitor, phenelzine (PHZ) 4 mg/kg/day in 2 divided doses for 14 days. The 1st week of PHZ treatment, this group received Veh; while the 2nd week of PHZ, the group received 5-MeODMT, 50 µg/kg. MAO inhibition was determined by assaying platelet MAO activity using ^{14}C-tyramine as substrate. By day 7 of PHZ, all PHZ-treated monkeys had at least 90% MAO inhibition with a mean ± SEM of 96.1% ±0.9%. Behavioral observation of the colony by an experienced, "blind" observer occurred for 1 hour, 5 days each week, beginning 5 mins. after injection. At 250 µg/kg, 5-MeODMT induced dog-like wet shakes, involuntary limb jerks, & hypervigilance while decreasing social groom & increasing submissive gestures in some treated animals. PHZ + 5-MeODMT 50 µg/kg increased wet shakes, limb jerks, & vigilance above levels seen with 50 µg/kg of 5-MeODMT alone. The combination also induced stereotyped behavior & reduced social grooming to a greater extent than 5-MeODMT alone. It appears that MAO inhibition potentiates at least some of the behavioral effects of 5-MeODMT. This suggests that 5-MeODMT may, at least in part, act through monoamine systems.

1441 PHARMACOLOGICAL KINDLING PRODUCED BY THE LOCAL ANESTHETIC-LIKE PESTICIDE, CHLORDIMEFORM. George K.W. Yim*, William R. Pfister* and Vickie Nolan*. (SPON: R.G. Babington). Dept. Pharmacology and Toxicology, Purdue University, West Lafayette, IN 47907.

Chlordimeform (CDM) has a number of actions common to local anesthetics (i.e. nerve conduction blockade, amygdaloid EEG seizure activity, convulsions, cardiovascular depression, etc.) and induces behavior characteristic of the serotonergic syndrome described by Jacobs (Life Sci. 19: 777-786, 1976). Since pharmacological kindling had been demonstrated with cocaine and lidocaine, the effects of subconvulsive doses of CDM were examined in rats. The daily administration of 60 mg/Kg CDM (i.p.) resulted in the progressive development (kindling) of seizures (100% response in 10 days). Spontaneous (pre drug) seizures also developed at a parallel rate with a 5 day lag period. This kindling phenomena was reversible; by fifteen days after drug cessation, only 50% of the animals showed spontaneous seizures. Although cropophagia and consumption and growth rates were decreased. These results suggest that pharmacological kindling is a phenomena common to convulsant local anesthetics. Besides the kindled seizures, a progressive development of tremor, spasticity, hyperreactivity and increased locomotor activity was also observed following the chronic 60 mg/Kg daily doses of CDM. In naive animals, these components of the serotonergic syndrome could only be obtained by higher single doses of CDM. The studies provide another example of pharmacologically kindled behavior. (Support in part by grants from NIH (NS 12077) and EPA (R-803965).

RECEPTORS

1442 SPECIFIC ^{3}H-MUSCIMOL BINDING TO SYNAPTIC GABA RECEPTORS. Beaumont, K.*, Chilton, W.*, Yamamura, H.I. and Enna, S.J., U. Arizona, Coll. Med., Tucson; U. Wash., Seattle; U. Texas Med. Schl., Houston.

Muscimol (3-hydroxy-5-aminoethylisoxazole) is a structural analog of γ-aminobutyric acid (GABA) and has been shown, both neurophysiologically and biochemically, to be a potent GABA receptor agonist. In man, after systemic administration, muscimol is soporific at low doses and hallucinogenic at high doses. Since it is possible that the behavioral effects illicited by this drug are partially due to an action at a receptor other than the GABA site, the characteristics of high affinity ^{3}H-muscimol binding were studied in brain to determine the specificity of this agent for the synaptic GABA receptor. Rat brain synaptic membranes (2 mg protein) were incubated for 15 min at 4° in 6 ml 0.05 M Tris-citrate buffer (pH 7.1 @ 4°) containing 1.5 nM custom tritiated muscimol (0.28 Ci/mmole) in the presence or absence of various concentrations of unlabeled compounds. The reaction was terminated by a 10 min centrifugation at 48,000 x g and, after rinsing, the pellet was dissolved and analyzed for radioactivity. Specific ^{3}H-muscimol binding was found to be saturable with a high affinity (K_d = 2.2nM). The regional distribution of ^{3}H-muscimol binding is strikingly similar to that reported for GABA receptor binding with the cerebellum > cerebrum > striatum > hippocampus > midbrain > hypothalamus > medulla-pons > spinal cord. ^{3}H-Muscimol binding is also pharmacologically identical to GABA receptor binding with only those drugs and amino acids which are known to neurophysiologically interact with the GABA receptor having any potency in displacing ^{3}H-muscimol. The binding is stereospecific with the neurophysiologically active isomers of GABA receptor agonists and antagonists having a greater affinity for the binding site than the inactive isomers. The results suggest that muscimol is a highly specific GABA receptor agonist which can be used to better understand the behavioral, biochemical and pharmacological characteristics of the GABAergic system. Supported by USPHS MH-27257 and an NIMH RCDA (HIY), and USPHS MH-29739, The Huntington Chorea Foundation, Pharmaceutical Manufacturers Association, and Merck Sharp & Dohme Co. (SJE).

1443 DIFFERENTIATION OF ANGIOTENSIN AGONISTS AND ANTAGONISTS AT MAMMALIAN BRAIN ANGIOTENSIN RECEPTORS. James P. Bennett, Jr. and Solomon H. Snyder. Dept. Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205

Under appropriate conditions [^{125}I]-(Ile$_5$) angiotensin II (^{125}I-AII) binds to mammalian brain membranes reversibly, and with high affinity (K_D=0.2 nM), and is displaced by unlabeled angiotensin peptides in a manner consistent with physiological interactions with angiotensin receptors (Bennett and Snyder, JBC 251:7423, 1976). ^{125}I-AII binding to brain and adrenal gland membranes is reduced 80-90% by decreasing the [Na^+]in the incubation buffer from 150 mM to 10 mM. Small cations (Na^+, Li^+, K^+) are more effective than larger cations (Rb^+, Cs^+, Hex^+, $(Et)_4N^+$) in reversing the decrease in ^{125}I-AII binding in 10 mM Na.

Sarcosine$_1$leucine$_8$angiotensin II (S_1L_8AII), a potent angiotensin antagonist, can be iodinated by the Chloramine-T procedure to s.a. of 600-700 Ci/mmol. ^{125}I-S_1L_8-AII binds to rat or calf brain and rabbit adrenal gland membranes reversibly and with high affinity (K_D=0.3 nM in calf brain). In 150 mM Na^+ buffer unlabeled angiotensin peptides displace ^{125}I-S_1L_8-AII binding with potencies very similar to their potencies at displacing ^{125}I-AII binding, suggesting that ^{125}I-S_1L_8-AII also labels physiologically relevant angiotensin receptors. Unlike ^{125}I-AII binding to brain and adrenal, ^{125}I-S_1L_8-AII binding is reduced only about 25% by decreasing the [Na^+] to 10 mM. The potencies of unlabeled angiotensin antagonists at displacing ^{125}I-S_1L_8-AII binding to brain receptors are reduced 2-4 fold by reducing the [Na^+] to 10 mM. In contrast, the potencies of unlabeled angiotensin agonists at reducing brain ^{125}I-S_1L_8-AII binding are reduced 20-50 fold by lowering the [Na^+] to 10 mM.

Saturation studies of ^{125}I-AII binding to calf brain membranes indicate that the binding affinity is reduced about 20-fold by decreasing the incubation buffer [Na^+] from 150 to 10 mM with no decrease in the number of binding sites. This reduction in ^{125}I-AII binding affinity results from a 20-fold decrease in the apparent association rate constant in 10 mM compared to 150 mM [Na^+] with little change in the apparent dissociation rate constant.

In summary, reducing the incubation buffer [Na^+] selectively reduces the affinity of angiotensin receptors in brain and adrenal for angiotensin agonists. Angiotensin receptor binding studies using ^{125}I-S_1L_8-AII can discriminate in vitro between agonists and angtagonists. These findings are consistent with the existence of distinct agonist- and antagonist-preferring conformations of brain and adrenal angiotensin receptors and a selective interaction of [Na^+] with the agonist-preferring conformation. (Supported by USPHS DA-00266, RSDA MH-33128 (SHS) and Fellowship DA-05055 (JPB)).

1444 *REGULATION OF NICOTINIC ACETYLCHOLINE RECEPTOR IN BRAIN.* *Ronald J. Bradley, Barbara J. Morley, Joan F. Lorden, George B. Brown and George E. Kemp*. Neurosciences Prog., Sch. Med., Univ. of Ala. B'ham., Birmingham, AL 35294.*

Nicotinic acetylcholine receptor (nAChR) has been identified in mammalian and avian brain by both in vivo and in vitro labelling of α-bungarotoxin. α-bungarotoxin binding is not uniformly distributed throughout the rat CNS and corresponds well with known cholinergic markers. The variables which regulate the concentration of these binding sites have not been studied.

We have investigated several methods which might alter nAChR concentration in brain, including cross-breeding, lesions, prolonged drug administration and dietary choline loading. C57BL and DBA inbred mouse strains are known to differ in their level of cholinergic markers and in their response to cholinergic drugs. We have dissected and biochemically investigated α-bungarotoxin binding in cortical, hippocampal, hypothalamic, and brainstem reticular formation samples from C57 and DBA parental strains, their F_1, F_2, B_1, and B_2 crosses and their reciprocal crosses. C57BL mice have a larger brain weight and a greater number of α-bungarotoxin binding sites in most areas which indicates a dominant mode of inheritance. Females of all crosses have a greater concentration of nAChR and a higher concentration of protein. Reciprocal crosses did not differ in protein or nAChR indicating that maternal effects are not a significant environmental variable. Lesions of areas containing high concentrations of nAChR produce increases in nAChR and protein levels in cholinoceptive areas.

We have not yet found a drug regimen which produces alteration of nAChR concentration, but administration of choline in the drinking water produces an increase in nAChR throughout the brain without a parallel change in protein level.

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1445 COMPARISON OF AGONIST AND ANTAGONIST BINDING TO β-ADRENERGIC RECEPTORS IN THE CEREBELLUM. David B. Bylund, David C.U'Prichard and Solomon H. Snyder. Dept. of Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205

The properties of the β-adrenergic receptors in the calf cerebellum have been studied using an antagonist ligand [^{3}H]dihydroalprenolol (DHA) and an agonist ligand [^{3}H]epinephrine (EPI). At 25°C, the specific binding of both ligands to a crude particulate fraction is rapid, reversible, and saturable. The binding is stereospecific with the (-)-isomers of both agonists and antagonists being 30-150 fold more potent than the corresponding (+)-isomers.

Ligand	Inhibition of Specific Binding IC_{50} nM [^{3}H]DHA	[^{3}H]EPI
(-)-isoproterenol	100	2
(-)-epinephrine	300	20
(-)-norepinephrine	5,000	200
(+)-isoproterenol	6,000	300
(-)-propranolol	1	2
(±)-butoxamine	3,000	12,000
(-)-practolol	15,000	7,000
(+)-propranolol	130	60

Epinephrine is much more potent than norepinephrine in inhibiting the specific binding of both radioactive ligands, which suggests that the receptors are of the β_2 type. Furthermore, the β_2 antagonist, butoxamine, is more potent than the β_1 antagonist, practolol, in inhibiting specific [^{3}H]DHA binding, although they are about equally effective agaonist [^{3}H]EPI binding. The IC_{50} values for the agonists are at least 10-fold lower in inhibiting agonist binding than in inhibiting antagonist binding. This observation indicates that the β-receptor may have agonist and antagonist conformations similar to the opiate, dopamine, α-adrenergic and serotonin receptors. On the other hand, in frog erythrocyte membranes agonists had equal potencies in inhibiting the binding of an agonist and an antagonist ligand to β-receptors (Lefkowitz and Williams, PNAS 74:515, 1977). In contrast to the cerebellum, the β-receptors in the cerebral cortex, as well as the other regions of the calf brain appear to be of the β_1 type and [^{3}H]EPI binding to these receptors cannot be demonstrated. The β-receptors in the cerebellum of several other species such as lamb, dog and rat are also found to be of the β_2 type when assayed using [^{3}H]DHA. There is a wide variation in the apparent number of cerebellar β-receptors with calf ≥ lamb > dog > rat. Under our conditions, [^{3}H]EPI binding to β-receptors is observed only in the calf and lamb. (Supported by NIH grants MH-05063 and MH-18501.)

1446 α-BUNGAROTOXIN BINDING TO CHICK SYMPATHETIC NEURONS Salvatore Carbonetto*, Douglas M. Fambrough, and Kenneth J. Muller, Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Md. 21210.

α-Bungarotoxin (α-BuTX) binds to primary cultures of chick sympathetic neurons as well as to chick sympathetic ganglia. Light autoradiographs of cultured neurons incubated with ^{125}I-α-BuTX show that the toxin binds specifically to neurons and that this binding is completely blocked by curare (10^{-4}M).

Chick sympathetic neurons in culture have acetylcholine (ACh) receptors and iontophoresis of acetylcholine onto neurons, as early as three days in culture, produces depolarizing responses in these cells. The ACh response is quickly blocked by curare (10^{-4}M) but can still be recorded in cultures treated with α-BuTX (1μg/ml). Curare also blocked ACh responses recorded in neurons that were pre-treated with α-BuTX. Extracellular recording from an in vitro preparation of the lumbosacral sympathetic chain of 18 to 20 day embryos revealed a "ganglionic potential" that was thought to be synaptically mediated because stimulation of the post-ganglionic nerve produced little or no response in the pre-ganglionic nerve and also because curare (10^{-5}M) reversibly blocked the response. Application of α-BuTX in concentrations up to 60μg/ml had no effect on the ganglionic potential, though it seems clear from autoradiographs that the drug can readily diffuse into the ganglion.

The α-BuTX binding molecule in chick sympathetic neurons is an integral membrane protein. Binding of α-BuTX to a detergent extracted membrane fraction of sympathetic ganglia is saturable and is blocked by d-tubocurarine. Characterization of this molecule has been difficult since α-BuTX binds reversibly to neurons, dissociationg with a half-time of 3.5 hrs at 23°C. However, when α-BuTX is crosslinked to its receptor with glutaraldehyde, dissociation is reduced to less than 10% for 24 hrs at 23°C. On sucrose gradients this "fixed" toxin-receptor complex cosediments with α-BuTX-ACh receptor complexes extracted from cultures of chick muscle (10s). Thus, the α-BuTX binding molecule in chick sympathetic neurons remains a candidate for the neuronal ACh receptor. However, the failure of α-BuTX to affect cholinergic responses in these cells weakens the case for this interpretation and suggests that caution be used in biochemical and anatomical studies of α-BuTX binding in the nervous system.

1447 MULTIPLICITY OF OPIATE RECEPTORS: IMPLICATIONS FOR METHODOLOGY IN THE OPIATE RECEPTOR BINDING ASSAY. E. E. Codd* & W. L. Byrne. Dept. Biochem. UTCHS, Memphis, TN 38163.

While opiates produce in vivo a multiplicity of effects (antinociception, hypothermia, respiratory depression, peristaltic diminution, etc.), the phenomena of cross-tolerance between opiates and the blockade of opiate action by specific antagonists have pointed to a common receptor site. Recently, however, evidence for receptor heterogeneity has been found: high doses of levorphanol failed to protect against naloxone-precipitated withdrawal (JPET, 182:189, 1972). Differences were found in opiate binding properties of narcotic receptors from guinea pig ileum and rat brain (Acta Pharm. Toxic., 37:211, 1975). Morphine and pentazocine appeared to act on separate receptors to produce their analgesic effects, but on similar receptors to produce their respiratory effects (Opiates and Endogenous Opioid Peptides, p.281, 1976). Three classes of opiate receptors have been distinguished by differential actions of classes of morphine-like and nalorphine-like drugs (JPET, 197:517, 1976). We report here evidence for multiple brain opiate receptors in an inbred strain of mice, C57BL/6J. According to an opiate receptor binding assay on a crude particulate preparation, there are more opiate antagonist than agonist receptors. Competition experiments studying the binding of 1nm ^{3}H-dihydromorphine (DHM), ^{3}H-morphine or ^{3}H-naltrexone in the presence of unlabeled morphine, naloxone or naltrexone show differences. Morphine competes more effectively with ^{3}H-DHM or ^{3}H-morphine, while naloxone competes more effectively with ^{3}H-naltrexone. These relationships also hold in the presence of 100mM Na^{+}. Heterogenity in the opiate receptor population calls into question the practice of using a fixed unlabeled control for nonspecific binding without regard for whether the labeled drug is an agonist or an antagonist. A case might be made for the use of the radiolabeled and unlabeled forms of the same drug (Acta Pharm. Toxic., 37:211, 1975), but what "displacer" would one use in competition experiments between two different drugs? The use of a large excess of unlabeled drug to eliminate "non-specific" binding has carried over into work on endorphins. But why should an opiate be used as a standard in defining the binding of the hypothesized endogenous ligand(s) for the receptor? Furthermore, our work (Prog. Neuro-Psychopharm, 1977) shows that double reciprocal plots of the inhibition of DHM or naltrexone by partially purified endorphin extrapolate to 100% inhibition of binding without correction for supposed "non-specific" binding. These results call for more limited and careful use of controls for presumed non-specific binding in assaying for the opiate receptor.

1448 DIFFERENTIAL LOCALIZATION OF DOPAMINE RECEPTOR ^{3}H-HALOPERIDOL BINDING AND ADENYLATE CYCLASE. Ian Creese, Robert Schwarcz*, Joseph T. Coyle and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205.

The characteristics of ^{3}H-haloperidol (HAL) binding to rat striatal membranes and of the dopamine-stimulated adenylate cyclase (DAC) in rat striatal homogenates suggest that both may be related to dopamine (DA) receptors. Both exhibit a similar agonist specificity with apomorphine and DA > norepinephrine >> isoproterenol and stereospecificity with respect to butaclamol antagonism. However, although neuroleptic antagonism of HAL binding correlates well with in vivo pharmacological activity as DA antagonists, the butyrophenone and related neuroleptics are too weak relative to the phenothiazines in antagonizing DAC. This different pharmacological profile suggests that HAL binding and DAC may not be identifying the same receptor sites.

Stereotaxic injection of kainic acid (KA) into rat striatum has been shown to lead to degeneration of both GABAergic and cholinergic neurones intrinsic to the striatum while sparing the DA input. Since pharmacological evidence suggests that DA receptors may be present on the intrinsic neurones, HAL binding and DAC were measured following unilateral striatal KA injections. Two days following injection DAC was reduced by 80-90% in the injected striatum compared to the uninjected side. However, HAL binding was reduced by only 20%. HAL binding continued to fall over 6 days post-lesion reaching a minimum of 40-50% of control by day 6. These decrements were maintained for at least 22 days. Enzymatic assays indicated about a 60% loss of choline acetyltransferase (CAT) and glutamic acid decarboxylase (GAD) but no reduction in tyrosine hydroxylase (TH) activity at this time. Since histological analysis indicated a severe loss of striatal neurones with a reaction gliosis most of the DAC is probably neuronally located. Since nigro-striatal 6-hydroxydopamine lesion or midbrain hemisection does not reduce HAL binding it would appear that the remaining HAL binding sites are not DA autoreceptors. Additional possible sites for HAL binding are DA receptors on presynaptic terminals of other neurones innervating the striatum, blood vessels or glial cells. Since a major striatal afferent pathway arises from the cortex, large unilateral cortical lesions were made by knife cuts or aspiration. Five days after such lesions HAL binding was reduced by 25-50% with little change in TH or GAD activity. DAC and CAT were reduced, perhaps indicating some trans-synaptic phenomena.

These results indicate that the DAC and HAL binding sites in the striatum may be located on different neuronal structures and that some HAL binding may represent presynaptic DA receptors. (Supported by DA-05328, MH-26654, MH-18501, NS-13586 and NIMH RCDA's to JTC and SHS.)

1449 CLONIDINE IS A POST-SYNAPTIC α-ADRENERGIC ANTAGONIST IN THE RAT PAROTID: CORRELATION OF [^{3}H] DHE BINDING AND K^{+} RELEASE. J. N. Davis, W. Maury*, and E. Hoyler*. VA Hospital and Duke University, Durham, NC 27705.

The rat parotid gland offers unique advantages for the study of post-synaptic α-adrenergic receptors. Preparations of dispersed parotid acinar cells have no apparent pre-synaptic elements and release K^{+} when exposed to α-adrenergic stimulation. α-Adrenergic membrane receptors can be measured in dispersed cells and membranes from the parotid with [^{3}H] dihydroergocryptine (DHE) radioligand binding. We studied the effect of clonidine on K^{+} release and DHE binding in the parotid. Clonidine was potent in displacing [^{3}H] DHE from membrane binding sites (K_D = 0.1 μM), but did not cause K^{+} release from dispersed cells even up to concentrations of 100 μM. Instead clonidine was a potent competitive antagonist of epinephrine-induced K^{+} release (pA_2 = 0.3 μM). Preliminary data suggest that naphazoline and ST 600, related imidazoles, are also α-antagonists in the parotid. These data show that the α-adrenergic blockade by clonidine results from an interaction with post-synaptic α-adrenergic membrane receptors. This is the first report of the α-adrenergic blocking action of clonidine in a peripheral tissue and suggests that parotid α-receptors may differ from other peripheral α-receptors. Since DHE binding sites in rat central nervous system resemble DHE binding in parotid, receptors with properties similar to parotid α-adrenergic receptors may be present in brain. Whether the central hypotensive action of clonidine results from its α-adrenergic agonist or antagonist properties remains to be determined.

1450 DEPRESSION OF CUTANEOUS MECHANORECEPTOR RESPONSE TO MECHANICAL STIMULATION BY SKIN COOLING. E. Djalali* (SPON: E. R. Perl). Dept. Physiology, Univ. North Carolina, Chapel Hill, NC 27514.

Activity of single cutaneous sensory units with myelinated afferent fibers was recorded from centrally-cut peripheral nerve of barbiturate-anesthetized cat with micropipette electrodes, in the fashion described by Burgess and Perl (J. Physiol. 190: 541-562, 1967). The sensory units were classified according to the criteria described by Burgess, Petit and Warren (J. Neurophysiol. 31: 833-848, 1968). When a stable recording from a low threshold mechanoreceptor was attained, its response to a standardized, effective stimulus, such as the rapid movement of a camel's hair brush across the receptive field, was averaged over 25 trials; repeated averages gave reproducible and stable values. The skin of the receptive field was then cooled by contact with a small thermode held at 0°C and the mechanical stimulation repeated. A total of 45 hair follicle receptors (predominantly G-1 type) and 30 "field" receptors have been tested with various degrees of transient or persistent cooling. All have shown substantial (50-75%) depression of the mechanically-induced response. This depression occurred whether or not the cutaneous cooling evoked a response. These results suggest that the common experience of decreased tactile perception and acuity in skin areas exposed to a cold environment probably has a peripheral receptor origin. (Supported by a grant from USPHS, NS 10321-06.)

1451 STATIC SENSITIVITIES OF SPINDLE Ia and II AFFERENTS IN NORMAL AND STEROID-INDUCED ATROPHIC MUSCLES. E. Eldred and B.R. Botterman*. Dept. of Anatomy and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

A comparison of the static sensitivity of Ia and II afferents of de-efferented medial gastrocnemius muscles in normal and steroid-treated male cats was undertaken with two objectives: 1) to determine on a statistically reliable basis how these afferents may differ in static responsiveness, and 2) to test for effect on spindle function of a form of atrophy free of the alteration in muscle length inherent in other models of atrophy. Regression lines calculated for firing rates attained during stepwise stretch of the muscle showed for both afferent types a high degree of correlation between rate and muscle lengthening beyond the length threshold for firing. This permited meaningful comparison of position sensitivities. These, normalized with respect to resting length of the muscle, were significantly greater for group II fibers (63.2 imp/sec/total passive length range) than for Ia afferents (41.3). Rates for group II fibers were also somewhat greater for spontaneously firing units, non-spontaneous units at their length thresholds, and all units at half and near-maximal steps in muscle lengthening. Thus, under these conditions of progressive stretch within the physiological range, group II discharge is at all lengths of the passive muscle slightly more than that of Ia afferents, the difference becoming somewhat greater at longer lengths. This pattern should be reflected in total volumes of inflow reaching the cord, since the numbers of Ia and II axons from this muscle is said to be equal.

In atrophic muscles of cats given triamcinolone (4 mg/kg) for 10-14 days the only clear differences from values in the control animals were greater proportions of Ia and II units firing spontaneously and somewhat smaller length thresholds. This indicates that atrophy to the extent of a 30 to 40% loss in muscle weight may have relatively little effect on static sensitivity of the spindles; it suggests also that effects of steroid treatment on sensory endings may be less than that shown to occur in motor endings.

1452 ALPHABUNGAROTOXIN RECEPTORS IN THE REGENERATING RETINOTECTAL SYSTEM OF GOLDFISH. Andrew Francis*, Nisson Schechter*, and M.S. Gazzaniga, Dept. Psychology and Long Island Research Institute and the Dept. of Psychiatry and Behavioral Science, SUNY Stony Brook, N.Y. 11794.

Regeneration of the crushed optic nerve in several species has been well-studied anatomically, behaviorally and electrophysiologically. The regeneration of the optic nerve and its reinnervation of the optic tectum in goldfish is an established model system for the study of this phenomenon. A large body of evidence exists showing that the tectum is an organized array of specific loci to which the optic nerve fibers specifically connect. In our efforts to understand the molecular mechanism of these events, we have followed the number of α-bungarotoxin (α BuTx) sensitive receptor sites in the goldfish optic tectum after optic nerve crush and during regeneration.

Right sided intraorbital nerve crush was performed on large (10-12 cm) common goldfish maintained on a standard diurnal cycle. After various survival periods freshly excised left and right optic tecta were individually homogenized in a 200 μl volume (10 mM sodium phosphate, 0.4 mM phenylmethyl sulfonyl fluoride, .013 mM dimethylformamide, 1 mM sodium EDTA, 0.02% sodium azide) at pH 7.2 using a glass micro tissue grinder.

Tissue homogenates were incubated in the presence of an excess of I-125 α BuTx (spec. act. 10^6 ci/mole) and counted after a centrifuge assay. All counts were corrected for nonspecific binding by subtraction of controls obtained in the presence of a several hundred-fold excess of noniodinated toxin. CPM per tectum or per mg of tectum were expressed as a ratio (left:right) in order to control for variations in tectal mass between fish.

The results show a sharp drop (at least 30%) in the concentration of α BuTx sites in the deafferented tectum at 6-10 days, followed by a correlated increase in receptor sites as vision returns. These data are not inconsistent with a chemoaffinity model of specific reconnection during regeneration. At the same time, it would appear that the number of sites depends on the presence of viable optic fibers, thereby suggesting that there is a dynamic interaction between regenerating fibers and presumed postsynaptic sites. This suggests that the chemoaffinity mechanism does not involve the regenerating fibers seeking particular static targets. In continuing studies, we are applying this technique to other retinotectal phenomena, such as compression, to gain insight into the actual synaptic changes in this system.

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1453 PRODUCTION AND REVERSAL OF THREE MANIFESTATIONS OF DOPAMINE RECEPTOR SUPERSENSITIVITY. Arnold J. Friedhoff, Helen Rosengarten, Kenneth Bonnet and Murray Alpert. Dept. Psychiatry, Sch. Med., NYU, New York, NY 10016.

We have found that chronic administration of haloperidol to rats followed by a washout period results in the production of increased stereotypic response to apomorphine, increased specific binding of dopamine to striatal tissue and increased activity of dopamine stimulated adenylate cyclase. Administration of l-dopa for 10 days to half the supersensitive group, followed by washout, resulted in reversal of all of the manifestations of supersensitivity. This effort presumably occurred by increasing the dopamine impinging on the receptor.

1454 AN α-BUNGAROTOXIN BINDING COMPONENT FROM DROSOPHILA MELANOGASTER. Janice I. Gepner*, Bent K. Schmidt-Nielsen*, Reid W. von Borstel*, E. J. Wolinsky* and Linda M. Hall. Dept. of Biology, MIT, Cambridge, Mass. 02139

An ^{125}I-α-bungarotoxin binding component from Drosophila melanogaster has been characterized in both particulate and solubilized crude extracts. This component has the properties expected of an acetylcholine receptor. The ^{125}I-α-bungarotoxin binding has been shown to be specific by three criteria: 1) it saturates for increasing concentrations of ^{125}I-α-bungarotoxin; 2) it shows the expected pharmacological specificity of a nicotinic acetylcholine receptor; and 3) it is localized to synaptic areas of the Drosophila central nervous system where acetylcholine is thought to be the neurotransmitter. Partial purification of this component has shown it is sedimented at 20,000 x g (20 minutes). Two procedures have been developed for screening for receptor mutants. In one method, nicotine-resistant mutants have been isolated and extracts of these mutants have been tested for altered ^{125}I-α-bungarotoxin binding properties. The second approach involves a screen for isoelectric focusing variants. Preliminary experiments have shown that the Drosophila receptor-toxin complex is slightly more basic than the Torpedo receptor-toxin complex.

1455 INCREASE IN THE DOPAMINE RELEASE FROM THE RABBIT CAROTID BODY IN VITRO WITH INCREASING HYPOXIA. C. Gonzalez* and S. J. Fidone. Dept. Physiol., Sch. Med., Univ. Utah, Salt Lake City, UT 84112.

It is now well documented that dopamine (DA) is the principal catecholamine contained in the Type I cells of the carotid body (c.b.), and it has been suggested that during natural stimulation release of DA from the Type I cells may function to modulate the activity of chemoreceptor nerve fibers which terminate upon these cells. In the experiments reported here, we measure directly the DA released by the rabbit c.b. in vitro and demonstrate that the rate of DA release increases with increasing levels of hypoxia. Further, we have studied the relationship between DA release and chemoreceptor impulse activity recorded from the carotid nerve.

C.b.'s with their attached nerves were removed from adult rabbits, cleaned and then incubated at 37°C for 2 hrs. in modified Tyrode's containing 40 μM ^{3}H-Tyrosine. After incubation, the c.b.'s were mounted in a specially-constructed temperature and humidity controlled chamber in which the c.b. rested in a tiny platinum wire basket. Carotid nerve action potentials were recorded with a suction electrode and counted with the aid of a window discriminator and digital counting system. Tyrode equilibrated with 100%, 50%, 20%, 10% and 0% O_2 in N_2 and containing 5.7 μM harmaline and 100 μM propylgallate (to prevent the enzymatic degradation of DA) was superfused over the c.b. and collected in vials containing a carrier mixture to prevent degradation of DA. The labelled DA was adsorbed on alumina, eluted with acetic acid, identified electrophoretically, combusted in a sample oxidizer and counted in a liquid scintillation counter.

During superfusion with 100% O_2, DA was released at a rate of 1-2picogrs./c.b./5 min. collection period. During superfusion with 50%, 20%, 10% and 0%, this resting rate of DA release was increased by factors of 1.25, 12, 30 and 50 times, respectively. The study of the relationship between DA release and chemoreceptor discharges is at an early stage, but it appears that with weak hypoxic stimuli there is an approximate proportional increase in DA release and impulse activity, while with stronger stimuli the increase in DA release exceeds the increase in impulse activity. This latter observation may result in part from spuriously low nerve impulse counts due to occlusion by overlap of impulses at high discharge rates. Also, we have found that the presence of MAO and COMT inhibitors attenuates the duration and frequency of the chemoreceptor impulse discharge to strong hypoxic stimuli.

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1456 REGULATION OF PHOSPHORYLATION OF THE ACETYLCHOLINE RECEPTOR. Adrienne S. Gordon* and Ivan Diamond. Dept. of Neurology, University of California Medical Center, San Francisco, CA 94143.

We have recently shown that at least one subunit of the acetylcholine receptor (AChR) is phosphorylated by an endogenous membrane protein kinase activity present in AChR-enriched membranes from Torpedo electric organ (Gordon, et al., Nature, in press). Phosphorylation of the AChR requires 100m M K^+ and is inhibited by carbachol (Gordon, et al., PNAS, 74:263 (1977)). We now present data further characterizing the phosphorylation of the AChR. There is an absolute requirement for K^+. NH_4^+ and Li^+cannot substitute for K^+. Na^+ inhibits phosphorylation at high concentrations. Phosphorylation requires the presence of Mg^{++}. Mn^{+2} and Zn^{+2} stimulate phosphorylation at low concentrations, but inhibit at higher concentrations. Ca^{+2} cannot substitute for Mg^{++}. At Ca^{+2} concentrations up to 20mM, there is no measurable phosphorylation. Phosphorylation of the AChR is not stimulated by either cAMP or cGMP at concentrations from 10^{-5} -10^{-8} M under a variety of incubation conditions. Data will also be presented on the effects of cholinergic agonists and antagonists on phosphorylation of the AChR.

This is the first demonstration that the AChR is phosphorylated by an endogenous membrane protein kinase. Phosphorylation of the AChR in electric organ membranes is specifically dependent on K^+ and Mg^{++} and may be regulated by cholinergic ligands. These results provide support for the hypothesis that phosphorylation of the AChR may be an important regulatory step in the response of the post-synaptic membrane to a nerve impulse.

1457 HORMONAL AND IONIC INFLUENCES ON α-NORADRENERGIC RECEPTORS IN MAMMALIAN CNS. David A. Greenberg, David C. U'Prichard and Solomon H. Snyder. Depts. of Pharmacology and Psychiatry, Johns Hopkins Univ. School of Medicine, Baltimore, MD. 21205

Techniques for labeling central α-noradrenergic receptors have recently been developed, using the α-agonists [^{3}H]clonidine, [^{3}H]-epinephrine and [^{3}H]norepinephrine, the α-antagonist [^{3}H]WB-4101, and the mixed agonist-antagonist [^{3}H]dihydroergokryptine (DHE). Since striking effects on the receptor binding of [^{3}H]opiates are observed in the presence of sodium ions, we have investigated the possibility of similar influences with respect to the α-receptor. A variety of monovalent cations exhibit differential effects on the binding of [^{3}H]α-agonist and antagonist ligands. NaCl at 150 mM reduces the binding of [^{3}H]agonists by about 80% without reducing binding of the [^{3}H]antagonist WB-4101. Other sodium salts affect α-receptor binding similarly while potassium and lithium salts are less potent, suggesting that the cation is responsible for the observed decrease in binding. Dixon plots of binding inhibition by NaCl characterize the principal effect as a reduction in the maximal number of α-receptor binding sites. Choline chloride also potently inhibits α-receptor binding, with [^{3}H]agonist ligands most profoundly affected. Binding of the mixed agonist-antagonist [^{3}H]DHE is inhibited to an extent intermediate between the effects on pure agonist and antagonist ligands. The differential influences of ions on [^{3}H]agonist and [^{3}H]antagonist binding sites lend further credence to the "two-site" model of α-receptor function.

The possible involvement of central α- and β-receptors in the apparent adrenergic imbalance of dysthyroid states has also been investigated by administration of thyroid hormones and antithyroid agents to adult and perinatal rats. Such treatment has been reported to produce alterations in myocardial α- and β-receptor binding (Ciaraldi and Marinetti, Fed. Proc. 36:916, 1977; Williams et al., Clin. Res. 25:458A, 1977). The effects of these manipulations on the binding properties of α-noradrenergic ligands and on the β-receptor marker [^{3}H]dihydroalprenolol in the brain will be discussed. (Supported by USPHS grants MH-18501, MH-33128, MH-05105 and DA-05052.)

1458 MODEL FOR TRANSMITTER-MEDIATED EXTRA-RETINAL PHOTOTRANSDUCTION IN APLYSIA CALIFORNICA. Ronald D. Grisell* and Michael C. Andresen* (SPON: F. H. Rudenberg). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX.

A three compartment, diffusion + reaction model has been developed for certain light sensitive cells in the central ganglia of the marine mollusc Aplysia californica. The R_2 giant neuron and the ventral photoreceptive cell respond to illumination with a graded hyperpolarization due to an increase in membrane permeability to potassium. Three morphological elements are considered: a lipochondrial compartment which contains the chromophore and acts as a transmitter source, a cytoplasmic compartment, and a compartment near the membrane which contains the transmitter-activated potassium conductance channels. Diffusion equations are defined for each compartment. Two chemical reactions are considered major factors in determining the final response kinetics. In the lipochondrial compartment a reaction rate equation governs a reuptake process of the transmitter and a binding reaction in the membrane compartment describes the interaction of the channels with the transmitter. The cytoplasmic compartment is assumed to serve as a diffusional conduit for the transmitter and to make no dynamic contribution to the response.

A fit of data at varying temperatures enable the model to predict that the rising phases of impulse and step responses are governed primarily by diffusion, whereas the falling phases are determined by reaction rate. These data also indicate a slight nonlinearity, which is included in the model as a bimolecularity of reaction, and which is further supported by good predictions of double impulse responses. Moreover, geometric parameters of the model allow comparison of R_2 and the VPN which have different radii.

1459 PHOTOAFFINITY LABELING OF NORADRENERGIC BINDING SITES WITH ^{3}H-1-NOREPINEPHRINE. Kim Allyn Heidenreich* and Edith D. Hendley. Dept. Physiol. & Biophys., Univ. Vermont Coll. Med., Burlington, VT 05401.

Catechol containing compounds are photosensitive to ultraviolet light (UV) and form covalent bonds with binding proteins in membrane preparations upon exposure to UV. Thus, the natural agonist, norepinephrine (NE), can serve directly as a photoaffinity label for macromolecules which bind it. Previous studies using catecholamine agonists suggest that they bind to a heterogeneous group of specific and nonspecific binding sites. Irreversible binding of NE allows for the use of very low concentrations of ligand, thus providing for selectivity of high affinity sites, and allows for gel electrophoresis of these binding proteins so that they can be separated and characterized. Synaptosomal membrane fractions of rat cerebral cortex were incubated with 1-2 nM ^{3}H-1-NE at 37°C for 10 min, then placed over ice in a cold room, and exposed to UV irradiation for various time periods. Following irradiation, half of the samples were incubated with nonradioactive 1 mM 1-NE to displace any radioactive ligand that did not bind covalently. Results indicated that the binding was irreversible after UV exposure. Total binding in samples exposed to UV was at least 10-fold greater than that in nonirradiated samples. At all times observed, binding was reversible in the absence of UV. The binding sites were saturable, and bound 0.247 pmoles ^{3}H-1-NE per mg of synaptosomal membrane protein. Simultaneous incubation of ^{3}H-1-NE with unlabeled 1-NE reduced covalent binding up to 90%. 1-NE at less than micromolar concentrations was more potent in displacing ^{3}H-1-NE than was d-NE. Covalent binding was inhibited by 60% with 0.1 mM phentolamine. At the same concentration binding was not inhibited by propranolol. Our results indicate that the photoaffinity labeling technique can be used to bind the natural agonist irreversibly to relevant noradrenergic binding sites. The pharmacological data suggest that a fraction of these binding sites may represent the alpha-adrenergic receptor in the rat cerebral cortex. We are further characterizing these binding proteins by SDS gel electrophoresis. Preliminary electrophoretic analysis indicated that the radioactive ligand bound selectively to a small fraction of the membrane proteins. (Supported by NIH grant # MH 25811).

1460 CHARACTERIZATION OF Na-INDEPENDENT (Na-I) and Na-DEPENDENT (Na-D) GABA BINDING TO BRAIN MEMBRANES. Robert Hitzemann*, Yasushi Ohizumi* and Horace Loh* (SPON: E. Callaway). Depts. Psychiatry and Pharmacology, University of Calif., San Francisco, CA 94143.

Enna and Snyder (Brain Res. 100: 81, 1975) have demonstrated that there are both Na-I and Na-D binding sites in brain membranes. These authors suggested that the Na-I binding is occurring to physiologically pertinent receptors while the Na-D binding is occurring to glial GABA transport sites. Using the high affinity transport of β-alanine (ala) into nerve endings (Hitzemann, Fed. Proc. 36: 968, 1977) as a model for glial GABA transport, we have attempted to examine the hypothesis of Enna and Snyder concerning Na-D binding. IC-50 values for β-ala transport and Na-D binding were determined for the following compounds: GABA, β-guanidopropionic acid, 3-OH-GABA, bicuculline, imidazole-4-acetic acid, trans-2-aminocyclopentanecarboxylic acid (ACPC), cis-3-ACPC, trans-3-ACPC, cis-3-aminocyclohexanecarboxylic acid, β-ala, strychnine, γ-guanidobutyric acid, 2,4-diaminobutyric acid, taurine, glycine, proline, α-ala and serine. The correlation coefficient between β-ala transport and Na-D binding was not significant (r = 0.04). Furthermore, we observed that the rank order of the various compounds to inhibit Na-D binding was similar to the rank order of their potency to produce GABA-like neurophysiological effects. Thus, we conclude that Na-D as well as Na-I binding may be occurring to pertinent receptors. The subcellular distribution of the Na-I and Na-D binding sites was examined. The Na-I sites were found to be associated with nerve ending membranes while the Na-D sites were found to be associated with microsomal membranes. The size of the Na-I sites in nerve ending membranes and the Na-D sites in microsomal membranes were characterized using cis-3-ACPC (amino-N to carboxy-C, 4.1 Å) and trans-3-ACPC (amino-N to carboxy-C, 4.8 Å). For Na-I binding the IC-50 values were cis-3-ACPC, 2.5×10^{-5} M, and trans-3-ACPC, 2.0×10^{-7} M. For Na-D binding the IC-50 values were cis-3-ACPC, 4.1×10^{-6} M and trans-3-ACPC, 1.6×10^{-6} M. These data suggest that the size of the Na-I site is greater than the Na-D site. Interestingly, these data are analogous to the situation reported by Nicoll (Brit. J. Pharmacol. 59: 303, 1977) for the depolarizing GABA receptor on primary afferents and the hyperpolarizing GABA receptor on motorneurons. (Supported in part by USPHS grants, DA-00564 and MH-25487.)

1461 CHARACTERIZATION AND ISOLATION OF SEROTONIN BINDING PROTEIN(S) IN RAT BRAIN BY AFFINITY CHROMATOGRAPHY AND GEL ELECTROPHORESIS. Louise L. Hsu* and Robert B. Chang* (SPON: John Doherty). Dept. Psych., Univ. Chicago, Chicago, IL. 60637

The serotonin (5-HT) affinity gel beads were synthesized according to the method of Cuatrecasas (J.B.C. 245: 3059, 1970) by coupling 5-HT with p-diazonium-benzamido-hexyl-Sepharose. The membrane (P_2D) and soluble(S) fractions of nerve endings were prepared from whole rat brains by the method of Gray and Whittaker (J. Anat. 96: 79, 1962) with slight modifications. Aliquots of the S and Triton X-100 treated P_2D fractions were incubated separately with 5-HT Sepharose gel beads in 0.025 M Na-phosphate buffer (pH 7.5) at 37°C for 20 minutes. At the end of incubations, the reaction mixtures were centrifuged at 20 K rpm for 20 minutes and the supernatants were separated. The beads were washed twice with phosphate buffer by vortexing and centrifuging at 20 K rpm. Then the presumed 5-HT-binding protein(s) were eluted from the gel beads with 10^{-5} M of d-LSD, l-LSD, methiothepin (MET) and cyproheptadine (CYP) consecutively. Disc gel electrophoresis revealed at least 5 protein bands at 1/3 of the gel length from the origin, a medium size band at 1/2 and 2 broader bands at 2/3 down the gel. Likewise, the MET and CYP eluates also showed the same 2 bands around 2/3 down the gel. Furthermore, the d-LSD eluate of the Triton X-100 treated P_2D fraction demonstrated 3 more bands beyond 2/3 down the gel and at least 2 more bands between origin and 1/3 down the gel, in addition to all the 5 bands observed in the d-LSD eluate of the S fraction. On the other hand, the l-LSD eluate of P_2D showed 1 extra band in the middle of the gel and also 3 bands beyond 2/3 down the gel. MET and CYP eluates showed similar bands as d-LSD did but a lot lighter. In separate experiments, the end supernatant fraction from whole brain (Se, the soluble fraction further separated from vesicles) was incubated with [C^{14}] 5-HT (10 nM) in the presence of pargyline at 37°C for 20 minutes and applied to polyacrylamide gels for electrophoretic analysis of both the protein and the [C^{14}] 5-HT binding patterns. At least three [C^{14}] 5-HT binding peaks were observed after gel electrophoresis. These data indicated that there might be multiple forms of 5-HT-binding protein in both the soluble and the membrane fractions of the nerve endings in rat brain.

1462 AGONIST OCCUPANCY OF α-ADRENERGIC RECEPTORS ON HUMAN PLATELET MEMBRANES INHIBITS CYCLIC AMP PRODUCTION. Marian S. Kafka, John F. Tallman and Craig C. Smith*. Adult Psychiat. Br., NIMH, Bethesda, MD 20014.

In the human platelet prostaglandin E_1 (PGE_1) activates adenylyl cyclase and increases cyclic AMP (cAMP) production. l-Norepinephrine (NE) inhibits PGE_1-stimulated cAMP production. Phentolamine, phenoxybenzamine, and dihydroergocryptine reverse the NE inhibition of PGE_1-stimulated cAMP production but propranolol and alprenolol do not, indicating that NE acts through an α-adrenergic mechanism. [^{3}H]dihydroergocryptine binds specifically to human platelet membranes. The binding is saturable and the single class of binding sites has a high affinity for [^{3}H]dihydroergocryptine. Binding is stereospecific as l-epinephrine (E) and NE potently inhibit binding, whereas d-norepinephrine is much less potent. The α-adrenergic antagonists phentolamine and phenoxybenzamine, and the α-adrenergic agonists NE and E, are potent inhibitors of binding whereas the β-adrenergic agonist isoproterenol and the β-adrenergic antagonist propranolol are weak inhibitors. The potencies with which the α-adrenergic agonists and antagonists inhibit PGE_1-stimulated cAMP production correlate well with the potencies with which these agents inhibit [^{3}H]dihydroergocryptine binding. NE and E inhibition seem to be mediated through a decrease in the apparent affinity for PGE_1 of the prostaglandin binding site which stimulates cAMP production. The relative percentage of NE inhibition is independent of temperature.

Platelet α-adrenergic receptors, occupied by agonists, inhibit PGE_1-stimulated cAMP production and may mediate a physiologically important early step in platelet aggregation. It is possible for the first time to assess directly α-adrenergic function in a variety of clinical conditions.

1463 CHARACTERIZATION OF AN α-BUNGAROTOXIN BINDING COMPONENT FROM CHICK BRAIN. C. Marchand*, G. Wang* and J. Schmidt* (SPON: W. Van der Kloot). Dept. Biochem., SUSB, Stony Brook, NY 11794.

Snake α-toxins, including α-bungarotoxin (α-BuTX), bind with high affinity and specificity to acetylcholine receptors (AcChR) in skeletal muscle and electric organ. α-BuTX has been found to bind to specific receptors in membrane preparations of the CNS as well, but so far physiological effects of this toxin on neuronal AcChRs have not been observed. In an attempt to elucidate the reasons for the central ineffectiveness of α-BuTX we have begun to analyze the interactions of receptor, toxin and cholinergic ligands in preparations from the CNS and skeletal muscle of the chick.

Newly-hatched chicks contain high levels of α-BuTX binding sites (G. Wang & J. Schmidt, Brain Res. 114, 524-529 (1976)) in their CNS, especially in the optic tectum and retina. Animals were sacrificed during their first four days after hatching, the optic lobes quickly removed and homogenized in phosphate buffer containing 1% of the nonionic detergent Triton-X-100. After centrifugation for 30' at 100,000 x g the supernatant was analyzed for toxin binding activity using the DEAE cellulose disk assay (J. Schmidt & M. A. Raftery, Analyt. Biochem. 52, 349-354 (1973)).

It was found that, whereas binding of α-BuTX to muscle extracts is irreversible, binding to CNS receptor is reversible, with a K_D of 2×10^{-10} M. The half life of the CNS receptor-α-BuTX complex is 2.3 hours, but is significantly reduced in the presence of the cholinergic ligands nicotine, acetylcholine, carbamylcholine and hexamethonium. The dissociation studies suggest that nicotinic ligands and α-BuTX bind to separate or partially separate sites; it is therefore conceivable that the central inefectiveness of α-BuTX is due to either the non-identity of the sites for toxin and neurotransmitter, or else to the rapid release of α-BuTX, induced by AcCh under in vivo conditions.

Chick brain homogenates were fractionated according to Gray & Whittaker (J. Anat. 96, 79-88 (1962)) and close to 40% of the α-BuTX binding activity recovered in the nerve ending fractions. After labelling with ^{125}I-α-BuTX synaptosomal membranes were reacted with the crosslinking agent suberimidate. SDS polyacrylamide gel electrophoresis of treated membranes followed by autoradiography revealed two radioactive bands with apparent molecular weights of 60,000 and 250,000 daltons respectively.

1464 A COMPARATIVE PHARMACOLOGICAL STUDY OF CAROTID BODY CHEMORECEPTORS. L. Monti-Bloch* and C. Eyzaguirre. Dept. Physiol., U. of Utah Col. Med., Salt Lake City, UT 84132.

Carotid bodies and their own nerves were excised from rabbits or cats, cleaned of surrounding connective tissue and placed in a chamber through which flowed mammalian saline equilibrated with different gas mixtures. pH was adjusted to 7.43 for the cat and to 7.4 for rabbit preparations using HEPES-NaOH buffer. Temperature was kept at 36-37°C. Single fibers were isolated, recorded from and identified as chemosensory by their response to hypoxia, hypercapnia, acidity and interruption of flow. Drugs dissolved in saline were applied upstream in volumes not exceeding 25 µl. In the rabbit, carbachol (1-10 µg) and ACh (1-50 µg) usually depressed the discharge and this was followed by discharge increase. Nicotine (1-20 µg) induced receptor stimulation in sigmoidal fashion with a linear relationship between 5 and 10 µg. Nicotinic stimulation was blocked by d-tubocurarine (10^{-6} g/l). Pilocarpine (1-20 µg) induced discharge depression following a negative sigmoidal curve. There was a negative linear relation between 2.5 and 10 µg. Pilocarpine-induced depression was blocked by atropine (10^{-6} g/l). Dopamine (1-100 µg) always increased chemoreceptor discharge also following a sigmoidal curve. The response was linear between 10 and 50 µg. In the cat, carbachol, ACh and nicotine (same doses as those used in rabbits) always induced chemoreceptor stimulation. Pilocarpine (up to 20 µg) had no effect on chemosensory discharges. Dopamine (1-200 µg) always depressed the discharge showing a sigmoidal curve with a negative slope. The response was linear between 10 and 100 µg. These experiments indicate that cat carotid body chemoreceptors only have nicotinic sites while rabbit receptors have both nicotinic and muscarinic sites. Nicotinic receptors appear to be excitatory while muscarinic ones seem to be inhibitory. These preparations may have excitatory dopamine receptors in rabbits and inhibitory ones in cats.

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1465 *REGIONAL DISTRIBUTION OF NICOTINIC ACETYLCHOLINE RECEPTOR IN RAT BRAIN.* *Barbara J. Morley, Joan F. Lorden, George B. Brown, George E. Kemp*, and Ronald J. Bradley. Neurosciences Prog., Sch. Med., Univ. of Ala. B'ham., Birmingham, AL 35294.*

Several studies have identified nicotinic acetylcholine receptor (nAChR) in the mammalian nervous system; however, a thorough and detailed description of the regional distribution of nAChR from brain is not available. Using biochemical assays and/or light autoradiography of labelled α-bungarotoxin, the following areas have been found to contain at least moderate amounts of nAChR: cortex, colliculi, olfactory tubercle, hippocampus, lateral geniculate nucleus, and hypothalamus, while the caudate and cerebellum contain neglible amounts of toxin binding.

When expressed as fm toxin per mg protein, the brainstem contains a high concentration of nAChR.

The present investigation was undertaken to extend the description of the regional distribution of nAChR in brain primarily to include several brainstem nuclei, utilizing a biochemical assay for ^{125}I-α-bungarotoxin. The following areas were found to contain significant amounts of nAChR (expressed as fm toxin/mg protein): cortex (52); hippocampus (50); hypothalamus (75); raphe (62); brainstem reticular formation (54); superior olivary complex (30); cochlear nucleus (44); inferior colliculus (69); olfactory bulbs (32); and olfactory tubercle (36). Areas containing little or neglible nAChR include: caudate (16); septum (15); and cerebellum (8).

The effect of several pharmacological agents on toxin binding to preparations from the various brain areas was also investigated. Nicotinic, but not muscarinic agonists and antagonists, were effective in blocking toxin binding. Decamethonium produced the greatest variability in toxin binding among brain preparations suggesting the possibility of a differing conformation of nAChR throughout brain. Mecamylamine, a ganglionic nicotinic blocker, was ineffective in blocking toxin binding in all prepartions, even at 10^{-4} M.

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1466 DOPAMINERGIC AND CHOLINERGIC RECEPTORS IN STRIATAL AND LIMBIC REGIONS AFTER CHRONIC HALOPERIDOL. P. Muller, J. Bowles* and P. Seeman, Department of Pharmacology, University of Toronto, Toronto, Canada.

It is known that chronic neuroleptic treatment produces an apparent dopaminergic supersensitivity in animals. In order to determine the biochemical basis of this supersensitivity, the amounts of dopaminergic and cholinergic receptors were measured in the limbic and striatal regions of rat brain after chronic treatment with haloperidol (10 mg/kg for over 6 weeks, given p.o.). Dopamine receptors were measured using either butaclamol-specific binding of ^{3}H-apomorphine (Seeman et al., Proc. Nat. Acad. Sci. 1976, 72, 4376) or of ^{3}H-haloperidol (Seeman et al., Nature 1976, 261, 717). Acetylcholine receptors were measured with ^{3}H-quinuclidinyl benzilate (Yamamura et al., Life Sci. 1976, 18, 685).

The chronic haloperidol treatment increased the specific binding of ^{3}H-haloperidol by about 40% in striatal homogenates and approximately 25% in mesolimbic tissue. The specific binding of ^{3}H-apomorphine also increased in both the striatum and the mesolimbic structures. The binding of ^{3}H-QNB was not altered in cortex, hippocampus mesolimbic areas and the striatum following the treatment.

The results suggest a degree of selectivity of chronic haloperidol on the receptor profile in different brain regions. (Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada).

1467 SUBUNIT STRUCTURE OF ACETYLCHOLINE RECEPTOR FROM RAT MUSCLE. Neil M. Nathanson*, Stanley G. Froehner*, Crispin Weinberg*, and Zach. W. Hall. Dept. Physiology, Sch. Med., UCSF, San Francisco, CA 94143.

Acetylcholine receptor from denervated rat leg muscle was purified 48,000-fold by affinity chromatography on concanavalin A-Sepharose and cobrotoxin-Sepharose. A control preparation containing only contaminants was made by performing a parallel purification with α-bungarotoxin added to the preparation prior to the cobrotoxin-Sepharose step. Comparison of the receptor and control preparations showed that the receptor was greater than 90% pure. Examination of the purified receptor by polyacrylamide gel electrophoresis in sodium dodecyl sulfate revealed two major polypeptide chains with apparent molecular weights of 45,000 and 51,000 daltons, along with minor components of 49,000, 56,000, 62,000 and 110,000 daltons. After alkylation of the receptor preparation with an affinity reagent for the acetylcholine binding site, 4-N-maleimidobenzyltri [^{3}H] methylammonium ion (^{3}H-MBTA), two peaks of radioactivity were obtained, with positions corresponding to molecular weights at 45,000 and 49,000 daltons. Radioactivity in both peaks was absent after prior incubation of the receptor with neurotoxin. To develop procedures for examining the small amount of junctional receptor in normal muscle, purified extrajunctional receptor was radioiodinated and further purified by sucrose gradient sedimentation. After electrophoresis in SDS and autoradiography, each of the polypeptide chains described above could be identified.

1468 CHEMORECEPTORS OF THE RAT KIDNEY. Giorgio M. Recordati‡ Nicholas G. Moss* and Linda E. Waselkov*. (SPON: D.L. Trevino). Depts. Physiol. and Med., University of North Carolina, Chapel Hill, N.C.

It is known that there are mechanosensitive nerve endings in the kidney, which respond to changes in arterial perfusion pressure, venous and ureteral pressures. Indirect observations suggest, however, that other kinds of receptors, such as chemoreceptors, might exist within the kidney. To investigate this possibility the electrical activity of afferent fibers has been recorded from the renal nerves of rats during experimental conditions which were designed to differentiate between mechanosensitive and chemosensitive endings. The experiments were carried out on male Sprague-Dawley rats, anesthetized with nembutal, paralyzed with gallamine and artificially ventilated. The right kidney was exposed through a paravertebral incision and the renal nerves were dissected from the renal hylus to the coeliac ganglion, decentralized and placed on bipolar hook electrodes. Twenty multifiber preparations (spontaneous activity of 0.2-3 imp/sec) were studied during renal ischemia produced by complete occlusion of the renal artery. In every instance the afferent activity began increasing 20 sec after the start of occlusion and reached a peak firing rate of 200-400 imp/sec after 40-60 sec of occlusion. The release of the renal artery occlusion was accompanied by a cessation of the discharge and was followed by a brief period of depressed spontaneous activity. The same multifiber preparations were also markedly activated after 60-120 sec of systemic hypoxia and hypercapnia produced by stopping the respirator pump. Single units which were sensitive to renal ischemia and systemic asphyxia were then selected in order to study their responses to changes in intrarenal pressure. Ten single units were unaffected by changes in arterial blood pressure in the range of 40-140mmHg produced by occlusion of the abdominal aorta below the renal arteries and by hemorrhage. All of them, however, were activated when the blood pressure was lowered below 30-40mmHg by an extreme hemorrhage. Increases in venous pressure, produced by occlusion of the inferior vena cava just above the level of the renal vein, caused an inhibition of three spontaneously active units. The same maneuver, when applied 30-60 sec after the start of a renal artery occlusion, was also effective in decreasing the activation, produced by renal ischemia, of four other units. In this case the deactivation of the units was accompanied by a visible backflow of venous blood into the pale cortex of the ischemic kidney. Increments in ureteral pressure amounting to 30-40cmH_2O were not accompanied by any consistent alterations in the discharge of four single units studied. These results indicate that the nerve endings which are sensitive to renal ischemia and systemic hypoxia and hypercapnia, might be defined as renal chemoreceptors.

1469 EFFECT OF CONTINUOUS PRESENCE OF ANTIBODY TO CHOLINERGIC RECEPTORS DURING SYNAPSE FORMATION IN VITRO. Rosemary P. Rees, Mohyee E. Eldefrawi* and Amira T. Eldefrawi*. LNNS, NINCDS, NIH, Bethesda, MD 20014 and Departments of Anatomy and Pharmacology, University of Maryland School of Medicine, Baltimore, MD 21201.

Preparations of nicotinic acetylcholine receptors purified from the electric organs of Torpedo ocellata were used to immunize rabbits. Receptor antibodies were detected by complement fixation with a titer of 1:320. The purified immunoglobulins contained 10 mg protein/ml estimated by the Lowry method. Application of rabbit serum, followed by labeling with goat anti-rabbit peroxidase conjugate resulted in patches of label on the surfaces of isolated rat superior cervical ganglion neurons. These patches were thought to be indicative of the location of acetylcholine receptors because no labeling was found in similar experiments using control rabbit serum. Cultures were also labeled at various times prior to and after synapses had been formed on their somata by neurites growing out from explants of rat thoracic spinal cord. Prior to contact by these neurites, patches of label were distributed unevenly over the neuronal somata. After synapse formation, labeling was heavy at synaptic clefts although some label was also present on neuronal somata away from synapses.

Cultures of superior cervical ganglion neurons combined with spinal cord explants were also grown in the presence of either serum or immunoglobulins diluted 1:10 with culture medium. Normal stages in synaptic development predominated for the first 48 hrs after neurites contacted ganglion cell somata. In older cultures, many examples of mature synapses were found which were not attached to ganglion cells. Early stages of synapse formation and incoming spinal cord neurites tipped by normal growth cones were also seen at the neuronal surface side-by-side with detached and degenerating synaptic boutons. None of these secondary changes were found in cultures grown in medium containing control serum. Thus, the presence of antibody to acetylcholine receptors does not appear to prevent initial stages of synapse formation, but does affect adhesivity and maintenance of mature synapses.

1470 ABSENCE OF NEGATIVE COOPERATIVITY OF BINDING OF NERVE GROWTH FACTOR TO EMBRYONIC CHICK SENSORY GANGLION CELLS. Richard J. Riopelle Ronald M. Harris-Warrick, Arne Sutter* and Eric M. Shooter. Dept. Neurobiol. Stanford Univ. Sch. Med. Stanford, CA 94305.

A number of studies have demonstrated specific nerve growth factor (NGF) binding to responsive tissues. Herrup and Shooter (PNAS 70,3384,1973) and Banerjee et al. (PNAS 70,2519,1973) using different tissue preparations found homogeneous saturable binding sites with dissociation constants of 10^{-10}M. Frazier et al. (J. Biol.Chem. 249,5513,1974) using homogenates of chick sensory and superior cervical ganglia detected multiple affinities of binding and presented evidence for cooperative interactions among receptor or ligand molecules. The question of site heterogeneity and cooperative interactions was reinvestigated using viable cell dissociates of 8-day-embryonic chick sensory ganglia and preparations of ^{125}I-βNGF which showed minimal non-specific binding (<5% of total). The binding of ^{125}I-βNGF revealed two distinct saturable binding sites with dissociation constants of K_d(I) : $2x10^{-11}$M and K_d(II) : $1.5x10^{-9}$M as measured by steady state and kinetic experiments. Ligand-ligand interactions do not complicate analysis of binding data because βNGF retains its dimeric character at concentrations below 10^{-12}M (M. Bothwell, unpublished data). The characteristics of the dissociation of ^{125}I-βNGF from sensory ganglion cells suggest that the binding sites I and II are distinct and do not result from intermolecular interactions of ligand or receptor. When dissociation is initiated by the addition of excess unlabelled βNGF to cells previously exposed to ^{125}I-βNGF, the dissociation pattern is biphasic. The ratio of the fast (K_{-1}(II) > $5x10^{-2}$ sec^{-1}) to slow (K_{-1}(I) = 10^{-3} sec^{-1}) dissociating components is dependent upon the concentration of ^{125}I-βNGF used to achieve steady state. This ratio varies according to the relative occupancy of the two sites as predicted from equilibrium binding data. The rates of dissociation of ^{125}I-βNGF following dilution into buffer alone or into buffer containing excess unlabelled βNGF was compared. For each concentration of ^{125}I-βNGF studied, there is a finite dilution factor above which the rate of dissociation of the ligand is the same whether or not unlabelled βNGF is present (100x at $4x10^{-12}$M; 1500x at $1.2x10^{-9}$M). By diluting labelled cells into buffer containing unlabelled cells it was possible to show that rebinding of ^{125}I-βNGF occurs at insufficient dilutions. Site heterogeneity is further supported by results showing that site I and site II are affected independently by different experimental conditions (A. Sutter et al., these proceedings). Supported by grants NINCDS NS04270, Deutsche Forschungsgemeinschaft, Medical Research Council of Canada.

1471 PEPTIDE ISOLATED FROM HUMAN BLOOD THAT INHIBITS N-METHYLTRANSFERASE ACTIVITY AND ETORPHINE BINDING. Helen Rosengarten, Marvin H. Lerner and A.J. Friedhoff Dept Psychiatry Sch. Med., NYU, New York, NY 10016.

We have previously demonstrated the presence of a low molecular weight peptide that inhibits both N-methyltransferase activity and etorphine binding to rat brain receptor (Abstract, meeting of American Society of Neurochemistry, Vancouver, March 1976; Pharm. Biochem. Behavior 5, supplement 1, p 147, 1976; and Life Sciences vol. 20, p 775, 1977). The apparent molecular weight of this peptide is 1500 and 1300. Here we present evidence that a similar peptide inhibitor of NMT activity and etorphine binding is also present in human red cells and plasma. The peptide is present in relatively high concentration in human blood, and IC_{50} is less than 10^{-7}M for both activities. This peptide may act as a regulator of N-methylation and a modulator of receptor function. The amino acid composition of the peptide has also been determined.

1472 COLCHICINE REVERSIBLY INHIBITS ELECTRICAL ACTIVITY IN ARTHROPOD MECHANORECEPTORS. Rollie Schafer, Paul D. Reagan, and Ngozi Okafo*. Dept. Biol. Sci., NTSU, Denton, TX 76203.

The dendrites of cockroach tibial spine mechanoreceptors contain hundreds of free microtubules. Could their presence be related to the generation of electrical activity by the receptor?

Deflection of a spine excites a single mechanoreceptor present the spine's base, producing a train of action potentials. The duration of the train and the frequency of spiking within the train are proportional to the strength and rate of mechanical stimulation.

Continuous perfusion of an excised leg over a period of 4 hr results in no response decrement. Perfusion with 10 mM colchicine decreases train length and spiking frequency within 5-7 min. The response reaches approximately one-fourth of its initial value by 20 min after the beginning of colchicine perfusion. The response returns to its initial level after reperfusion of the inhibited leg with perfusion solution lacking colchicine. The time courses of inhibition and recovery are comparable.

Irreversible inhibition is produced by perfusion with 1 mM vinblastine sulfate in perfusion solution containing 1% dimethyl sulfoxide. Control perfusion with 1% DMSO produces no effect. Perfusion solution containing 70% deuterium oxide is inhibitory. Deuterium oxide does not inhibit over short time spans at concentrations of less than 50%, nor does it counteract inhibition by 10 mM colchicine.

Overnight incubation in 10 mM colchicine does not inhibit axonal conduction in desheathed crab leg nerves or frog sciatic nerves. Thus, by analogy, the colchicine effect on cockroach mechanoreceptors is probably an effect on the sensory dendrites or soma. Colchicine may be affecting (1) intracellular microtubules, (2) membrane-associated tubulin, (3) other membrane components through nonspecific binding, or (4) axoplasmic transport of essential materials to the sensory dendrites.

1473 OPIATE RECEPTOR LIGAND IN CEREBROSPINAL FLUID: A SIMPLE RADIORECEPTOR ASSAY REQUIRING NO PRELIMINARY PURIFICATION. Haruo Shibuya*, Donald L. Bowie*, and Candace B. Pert. Sect. on Biochem., Adult Psychiat. Br., NIMH, Bethesda, MD 20014.

Based upon the ability to displace [^{3}H]dihydromorphine binding to opiate receptors, Terenius and Wahlström[1] have reported that purified, concentrated extracts of human cerebrospinal fluid (CSF) contain morphine-like material. We report a very sensitive and specific radioreceptor assay procedure which detects opiate peptides in the .05 pmole range. This enhanced sensitivity conveniently makes analysis of "raw," unextracted CSF possible.

[^{3}H]-D-Ala^2-met-enkephalinamide (47 Ci/mmole), an enzyme-resistant enkephalin analog,[2] binds with high affinity (0.1 nM) to a site on rat brain membranes which appears in the presence of $MnCl_2$ and a warm (25-37°C) incubation temperature.[3] Displacement of [^{3}H]-D-Ala^2-met-enkephalinamide from this Mn and temperature-dependent site, which is highly and specifically sensitive to opiate peptides, provides the basis of a radioreceptor assay which requires 100 µl aliquots of "raw" CSF.

To determine the molecular weight of the CSF material, lyophilized monkey lumbar CSF was applied to a calibrated Sephadex column (G-25, 2 x 40 cm) and eluted with 1% acetic acid containing 0.1% BSA. Analysis of the neutralized fractions revealed a single peak of molecular weight 1200-1500. This peak did not coincide with the very large peak of UV_{280} absorption which emerged in the void volume, nor the fractions where a [^{3}H]-met-enkephalin standard emerged. The opiate ligand in CSF does not cross-react in a radioimmunoassay which recognizes leu-enkephalin and, to a 5-fold lesser extent, met-enkephalin. Opiate ligand activity can be completely absorbed out of CSF by a single passage through an XAD-2 Amberlite column. The opiate ligand in CSF, which is resistant to inactivation by incubation with brain membranes, is certainly neither met- nor leu-enkephalin, and has a smaller molecular weight than C-fragment (β-LPH-61-91).

In preliminary experiments with lumbar CSF from drug-free patients, the morphine-equivalent opiate receptor ligand content (pmoles/.1 ml CSF ±SD) of 5 schizophrenics, 5 depressed patients and 9 personality disordered individuals were respectively 4.4 ±2.1, 4.3 ±2.1 and 3.6 ±5.9. Serial samples of ventricular monkey CSF contain gradually altering opiate ligand content which fluctuate in a broad range from 0 to 15 pmoles/.1 ml CSF and appear unrelated to episodes of repeated footshock.[4]

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1474 BIOCHEMICAL PROPERTY OF SEROTONIN-BINDING PROTEIN.
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Using 5HT-affinity chromatography, two serotonin-binding proteins (SBP) may be isolated from rat brain synaptosomal membrane. These membrane proteins were solubilized by 0.1% Emolphogen. One of them was eluted from the column by 10^{-4}M chlorimipramine (CIP), the other one was eluted by 10^{-4}M Lysergic Acid (LSD). Similar protein fraction may be obtained by eluting the column with 10^{-6}M CIP or 10^{-6}M LSD. Under appropriate condition, each of the four concentrated protein fraction (10^{-6}M, 10^{-4}M LSD eluted protein fraction and 10^{-6}M, 10^{-4}M CIP eluted protein fraction) revealed one protein band on SDS-gel electrophoresis, their molecular weight were about the same. When 5HT-affinity column was eluted by 10^{-6}M or 10^{-4}M 5HT instead of 10^{-6}M or 10^{-4}M LSD. Similar protein band was obtained. This result again suggested that LSD binding to SBP might be replaceable by 5HT.

10^{-6}M and 10^{-4}M LSD eluted protein (LSD-SBP) were pooled for the following studies. Approximately 1-2 µg purified LSD-SBP might be obtained from 1g (net weight) of rat brain. The purification was achieved for about 816 fold.

When purified LSD-SBP was incubated with 10^{-7}M H^3-5HT in 0.01M Tris-HCl buffer 7.4 with 0.1% Ascorbic acid at 37°C for 10 min. Significant H^3 label is detected in the protein fractions separated by gel filtration. If bovine serum albumin was incubated with H^3-5HT under the same condition, No H^3 radioactivity was detectable in the protein fractions. Furthermore, H^3-5HT binding to SBP was saturable (H^3-5HT concentration increased from 10 to 600 nM). By scatchard plot, two binding sites were observed. n_1 = 30 p mole/mg, kd_1 = 855nM
n_2 = 4.0 p mole/mg, kd_2 = 25nM
Since 10^{-6}M and 10^{-4}M LSD eluted protein were pooled for these experiments, whether the two different binding sites were contributed by the same SBP or different protein with similar molecular weight is not clear at the present time. (Supported by graduate faculty research support and Eli Lilly and Company).

1475 CHARACTERIZATION OF TWO DISTINCT CLASSES OF HIGH AFFINITY BINDING SITES FOR NERVE GROWTH FACTOR ON SENSORY GANGLIA CELLS FROM CHICK EMBRYOS.
Arne Sutter*, Richard J. Riopelle, Ronald M. Harris-Warrick and Eric M. Shooter. Dept. Neurobiol., Stanford Univ. Sch. Med., Stanford, CA 94305.

The binding of nerve growth factor (βNGF) to viable cell dissociates from sensory ganglia of 8-day-old chick embryos was reinvestigated using ^{125}I-βNGF preparations which showed minimal non-specific binding (<5% of total binding). The analysis of steady-state and kinetic data revealed two distinct saturable binding sites with dissociation constants of K_d(I) = 2 x 10^{-11}M and K_d(II) = 1.5 x 10^{-9}M. The ratio of the number of type I to type II binding sites is 1:5. At 37°C the binding characteristics of site I were studied in steady-state as well as in kinetic experiments and the data were in concordance. From association rate measurements at βNGF concentrations less than 2 x 10^{-11}M the rate constant k_{+1} (I) of 2 x 10^7 L Mol^{-1} sec^{-1} was determined. The dissociation rate constant for site I is k_{-1} (I) = 10^{-3} sec^{-1}. Numerous sets of data show that the two sites are distinct and not the result of cooperative interactions of ligand or receptor (R. Riopelle et al., these proceedings). In order to determine whether heterogeneity of the βNGF binding is due to the heterogeneity of the cell populations in the ganglia dissociate, neuronal cells were separated from non-neuronal cells by means of their decreased ability to adsorb to plastic culture dishes. Binding of βNGF to site I could not be detected on the non-neuronal cells, whereas the neuronal cell population was enriched in this high affinity binding. Single cell bioassay results and data from developmental studies support the hypothesis that binding to the high affinity site (I) is required for neuronal differentiation. The half maximal stimulation of neurite outgrowth from single cells by βNGF occurs at 10% occupancy of site (I) and only 0.2% occupancy of site (II). Preparations of βNGF modified by carboxy-terminal arginine cleavage which show unaltered affinity for site (I) but a 10-fold reduced affinity for site (II) in binding assays are as effective as native NGF in the single cell bioassay. The development of sites I and II was analyzed at various embryonic stages. Binding to site II is measurable at all stages studied. However, site I binding is not present at embryonic day 4, becomes measurable at embryonic day 6 and increases up to embryonic day 8. The appearance of site I during embryonic development correlates well with the period during which mediodorsal neurons, the putative target cells of NGF within the sensory ganglia, undergo their final division and begin to differentiate. Supported by grants NINCDS NS04270, Deutsche Forschungsgemeinschaft, Medical Research Council of Canada.

1476 CONFORMATIONAL REQUIREMENTS FOR THE ^{3}H-APOMORPHINE RECEPTOR: STEREOSPECIFIC BINDING OF 2-AMINOTETRALIN DERIVATIVES.
J.L. Tedesco, J. Bowles*, J.D. McDermed* and P. Seeman. Pharmacology Department, University of Toronto, Toronto, Canada.

Certain hydroxylated derivatives of 2-aminotetralins stimulate dopaminergic systems apparently by acting as dopamine receptor agonists. At very low doses they can induce stereotypy and/or emesis (McDermed et al., J. Med. Chem. 18, 362, 1975). They are relatively rigid analogues of dopamine in various conformations.

Competition for ^{3}H-apomorphine (3.5 nM) binding sites in calf caudate crude homogenate was tested with the above derivatives. Both butaclamol-stereospecific (124 fmoles/mg protein, K_D = 3.5 nM; Seeman et al., P.N.A.S. 73, 4354, 1976) and apomorphine-specific sites (250 fmoles/mg protein, K_D = 1.4 nM) have been demonstrated. We now report:

1. The (-)-enantiomer of 5-hydroxy-N,N-dipropyl-2-aminotetralin (or 5-OH-AT) had an IC_{50} of 18 nM, having 19-fold more potency than (+)-5-OH-AT in competing for specific or butaclamol-stereospecific ^{3}H-apomorphine sites.
2. Scatchard analyses show virtually identical densities of ^{3}H-apomorphine receptors stereospecific to either 70 nM (-)- and (+)-5-OH-AT or to 1 µM (+)- and (-)-butaclamol (124 and 110 fmoles/mg protein respectively). The respective dissociation constants (5.2 nM and 3.5 nM) were also similar.
3. The IC_{50} for (±)-5-OH-AT was 16 nM, having 35-fold greater potency than (±)-7-OH-AT and 60-fold greater than (±)-6-OH-AT.
4. All 6,7-dihydroxy-2-aminotetralin derivatives used were potent competitors for ^{3}H-apomorphine receptors regardless of the degree of N-alkylation (up to N,N-dipropyl substitution).
5. The 5,6-dihydroxy-2-aminotetralin derivatives showed highest affinity as N-methyl, N,N-dimethyl or N,N-diethyl derivatives (IC_{50} values of 11, 12 and 7 nM). They almost invariably showed from 3 to 60 times less affinity than did their 6,7-dihydroxy counterparts.

(±)-5-OH-AT should be more potent than (±)-7-OH-AT in competing for ^{3}H-apomorphine binding since the former shares the same dopamine-moiety conformation with apomorphine. It is surprising that the 5,6-dihydroxy derivatives were generally less potent than the 6,7-dihydroxy derivatives. This could suggest the existence of two high-affinity apomorphine receptors.
(Supported by Ontario Mental Health Foundation and Medical Research Council of Canada).

1477 ALTERED INSULIN BINDING TO CIRCULATING MONONUCLEAR CELLS IN MYOTONIA DYSTROPHIA.
Gerald J.M. Tevaarwerk*, Kenneth P. Strickland*, Chao-Hsiung Lin* and Arthur J. Hudson. Depts. Med., Biochem. and Clin. Neurol. Sci., UWO, London, Ont. N6A 5A5

Peripheral insulin resistance is an integral feature of myotonia dystrophica (MD) but the cause is unknown (Tevaarwerk and Hudson, J. Clin. Endocrinol. Metab. 44:491,1977). In the current study insulin resistance was investigated by comparing insulin binding to circulating mononuclear cells from MD and normal subjects. Mononuclear cells from 12 fasting MD patients and 12 age and weight-matched normal control subjects were isolated by gradient centrifugation of 300 ml of whole blood in a Ficoll-Hypaque mixture (Boyum, J. Clin. Lab. Invest. 21, Suppl. 97, 51, 1968). The binding assay was done according to the procedure of Schwartz et al (Proc. Nat. Acad. Sci. 72:474,1975) using ^{125}I monocomponent insulin alone and in the presence of gradually increasing amounts of unlabeled insulin. Binding was expressed as a percentage of the total ^{125}I-insulin that was bound per 10^6 monocytes. Basal serum insulin levels in the MD and control groups were measured by radioimmunoassay.

The mean (± SEM) insulin binding was 0.65 ± 0.10% in the MD subjects as compared with 1.26 ± 0.08% per 10^6 monocytes in the normal group. The addition of unlabeled insulin decreased labeled insulin binding to 0.35 ± 0.04% and 0.84 ± 0.13% per 10^6 monocytes for the MD and normal groups, respectively. The decrease in the normal group is significant ($p < 0.005$) but the initial low binding in the MD group prevents accurate measurement of inhibition of binding by the unlabeled insulin. From these results it is concluded that there is significantly decreased insulin binding to circulating mononuclear cells in MD. The decrease in binding in MD may be due to a reduced number of insulin receptors per cell or to a decrease in receptor affinity. The mean (± SEM) basal insulin levels were 19.8 ± 4.8 µU/ml in MD as compared with 8.2 ± 0.9 uU/ml in normal subjects ($p < 0.05$). The increased basal insulin level may be responsible for the decrease in binding; alternatively, both may be different manifestations of an as yet unrecognized mechanism producing insulin resistance in MD.

(Supported by MRC grant MA-5702.)

1478 α-BUNGAROTOXIN BINDING IN THE BRAIN OF THE HORSESHOE CRAB, LIMULUS POLYPHEMUS. William E. Thomas* and James G. Townsel (Spon: John S. Thomas), Departments of Biochemistry and Physiology, Meharry Medical College, Nashville, Tennessee 37208.

Acetylcholine (ACh) has been shown to be a sensory neurotransmitter in certain invertebrate species (Florey, 1973, J. Comp. Physiol. 83: 1; Sorenson, 1973, Biol. Bull. 144: 180; Hildebrand, et al., 1974, J. Neurochem. 23: 951). Recent investigation of the ventral nerve cord of Limulus has revealed the presence of significant levels of the ACh biosynthetic enzyme, choline acetyltransferase (Malthe-Sorenssen and Emson, 1976, J. Neurochem. 27: 341). These investigators suggest that ACh may serve as a sensory transmitter in Limulus. If ACh is a sensory transmitter in Limulus; then, the central nervous system should contain cholinoceptive cells. The nicotinic cholinergic receptor (AChR) is a reliable indicator of nicotinic cholinoceptive cells. The AChR can be identified by its affinity for the α-neurotoxins. Thus, we have initiated studies on the binding of (^{125}I) α-bungarotoxin to membrane fragments prepared from Limulus brain tissue.

In this tissue, toxin binding saturates in the range of 25 to 30 nM with maximum binding of 3-8 pmoles per mg of protein. The binding has a $t_{½}$ of 60sec., which yields a K_1 of 3.38 x 10^5 M^{-1} s^{-1}. Pharmacological studies show that binding is inhibited by both cholinergic agonists and antagonists. I_{50}'s for inhibition by d-tubocurarine, decamethonium, carbachol and atropine are 6 x 10^{-7}M, 10^{-5}M, 10^{-4}M, and 8 x 10^{-5}M, respectively. The saturation kinetics appear consistant with toxin binding to multiple sites. Moreover, the rate of dissociation of bound toxin indicates the presence of more than one species of toxin binding components. Toxin binding activity was solubilized with Triton X-100. Velocity sedimentation studies of this solubilized activity were performed on linear sucrose gradients (5-20%). Three separate components were detected with sedimentation coefficients of 8s, 15s, and 16.8s. These results are consistent with the suggested sensory transmitter function of ACh in Limulus.

(Supported by NIH Grants No. RR08037 and H1 17370)

1479 EFFECTS OF PROTEIN REAGENTS ON GAMMA-AMINOBUTYRIC ACID RECEPTOR-IONOPHORES IN CRAYFISH MUSCLE AND RAT BRAIN. M.K. Ticku* and R.W. Olsen. Dept.Biochemistry,Univ.Calif.Riverside, CA 92521.

The functional groups involved directly or indirectly in action of the inhibitory neurotransmitter γ-aminobutyric acid(GABA) are of interest in understanding receptor-ionophore function at the molecular level. Reagents for specific amino acid residues were found to inhibit the postsynaptic response to GABA in crayfish muscle and also to inhibit GABA receptor binding in crayfish muscle and mammalian brain.The physiological postsynaptic response to GABA involves an increased chloride conductance, which is inhibited noncompetitively by the convulsant drug picrotoxin (Takeuchi & Takeuchi(1969)J.Physiol.205,377).The anion selectivity and pH dependence(greater conductance on the acid side of neutrality) suggest that the GABA-regulated Cl^- ionophore contains a fixed + charge(Takeuchi & Takeuchi(1967)J.Physiol.191,575),possibly a protonated histidine.GABA was previously shown to cause a picrotoxin-sensitive specific increase in permeability to radioactive Cl^- in crayfish abdominal muscle(Ticku & Olsen(1977)Biochim.Biophys.Acta 464,519).This postsynaptic response to GABA,but not background Cl^- flux,was inhibited by preincubation of the tissue for 20 min at pH 6.0(22°)with 0.1-1 mM diethyl pyrocarbonate(DEP),reagent which covalently modifies primarily histidine residues under these conditions. Sulfhydryl group reagents such as N-ethyl maleimide(NEM) did not significantly inhibit the synaptic GABA response,although inhibiting GABA transport in this tissue(Olsen et al,(1975)Mol. Pharm.11,566).Radioactive GABA binding sites having the properties of postsynaptic receptor sites were measured in membrane fractions of crayfish muscle(Meiners et al.(1977)Fed.Proc.36,801)and rat brain(Olsen & Greenlee(1976)Fed.Proc.35,1947).These GABA receptor binding sites were inhibited by 1mM DEP at pH 6,consistent with DEP block of GABA synapses, and a possible role of a histidine residue in the binding of GABA to its recognition site(receptor)or subsequent GABA receptor-regulated Cl^- flux.Binding of GABA to nonreceptor,sodium-dependent uptake-binding sites in crayfish muscle was not affected by DEP,but was inhibited by sulfhydryl reagents. Picrotoxinin at 0.2 mM did not inhibit GABA binding to presumed receptor sites.A biologically active analogue, α-dihydropicrotoxinin(dHP),was made radioactive and found to bind to a limited number of saturable sites in postsynaptic membrane fractions of crayfish muscle and rat brain which appear to be related to picrotoxin's physiological site of action in blocking GABA synapses(Ticku(1977)Fed.Proc.36,751).GABA did not inhibit dHP binding in either tissue, nor did the protein reagents DEP or NEM. This supports the idea that picrotoxinin inhibits GABA synapses at a site distinct from the GABA receptor, perhaps allosterically.

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1480 BIOCHEMICAL CHARACTERIZATION OF BRAIN NICOTINIC CHOLINERGIC RECEPTOR. Richard S. A. Tindall, Dept. Neurology, Southwestern Med. Sch., UTHSCD, Dallas, Texas 75235

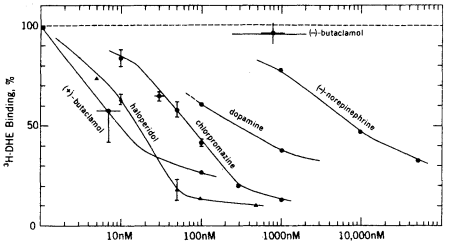
Biochemical assays of CNS acetylcholine receptors have demonstrated two types to be present; nicotinic and muscarinic. Employing an affinity ligand binding assay utilizing the iodinated nicotinic ligand alpha-Bungarotoxin (^{125}I-αBGT), we have determined the concentration of nicotinic acetylcholine receptors (nAChR) in bovine CNS structures. Toxin binding is present diffusely in cortex and subcortical nuclei, with greatest concentration in limbic structures. These data will be presented. Striatal structures known to possess high concentration of muscarinic receptor demonstrate low toxin binding (nAChR concentration). The distribution of nicotinic and muscarinic receptors differ as demonstrated by ligand assays.

Partial purification of CNS nAChR from bovine brain has been carried out. Cerebral cortices were homogenized in 0.32M sucrose and the P_2 (crude mitochondrial) fraction suspended in 2% Triton X-100, 0.05M sodium phosphate buffer p7.4, with PMSF present. After overnight stirring and centrifugation at 105,000 xg for 60 min., the supernatant (triton extract) was applied to an affinity column composed of the α-neurotoxin of Naja naja kaouthia coupled by cyanogen bromide to Sepharose 4-B. The column was then washed with 0.5% Triton-X-100 in phosphate buffer, and elution of the receptor from the affinity column carried out by incubating with 1mM benzoquinonium chloride in 0.1% Triton X-100 in phosphate buffer. The eluted fraction was concentrated, applied to a sucrose gradient (5-20% in 0.1% Triton X-100 in phosphate buffer and sedimented for 21 hours at 37,000 RPM (SW 41 rotor). Following fractionation, the protein peak was pooled, concentrated and aliquots were assayed for ^{125}I-αBGT binding.

Samples were reduced and dissociated (2-ME) and electrophoresed on poly-acrylamide gels containing SDS. The components of our nAChR preparation as so determined will be presented.

1481 BRAIN DOPAMINE RECEPTOR BINDING OF ^{3}H-DIHYDROERGOCRYPTINE. M. Tittler*, P. Weinrich* and P. Seeman, Pharmacology Department, University of Toronto, Toronto, Canada.

Although ^{3}H-DHE (dihydroergocryptine) binds to pituitary dopamine receptors (Caron et al., Fed. Proc., 1977), this ligand also binds to α-adrenergic receptors in the brain. In order to use ^{3}H-DHE as a ligand for brain dopamine receptors, we measured ^{3}H-DHE binding in the presence of 500 nM phentolamine, a concentration which saturates α-receptors but does not affect dopamine receptors. The K_D was 0.7 mM (calf caudate homogenate).



The inhibitory concentrations for various drugs (IC_{50} values in Fig.) are similar to those which inhibit ^{3}H-haloperidol binding (Nature 261, 717, 1976) in the striatum, and to those which inhibit ^{3}H-DHE binding in the pituitary (Caron et al.). Caudate and pituitary dopamine receptors are thus very similar.

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1482 ENDOGENOUS CORTICOSTERONE (B) IN INTACT STRESSED RATS: REGIONAL BRAIN CYTOSOL BINDING. Barbara B. Turner, Jerry Schielke* and Bernard J. Carroll*. Mental Health Research Health Research Institute, University of Michigan, Ann Arbor, Michigan, 48109.

Previous studies on the brain content of glucocorticoids have used either adrenalectomized (ADX) animals injected with 3H-B or total B levels in the brains of non-perfused, intact animals. We have examined the brain distribution of endogenous B cytosol binding following stress in the intact rat. Bound B, total B and the number of unoccupied sites, was assessed in 8 and 3 brain regions respectively in stressed and ADX adult male rats. At 1000h after 30 min. shaker stress, atrial blood was withdrawn for plasma B determination and the rats perfused. Brain regions from 4-5 animals were pooled and homogenized (20 ml/g) in Tris-EDTA buffer, pH 7.4. Free steroid was removed by dextran-charcoal. All samples were extracted into CH_2CL_2, chromatographed on LH-20, and B levels measured by radioimmunoassay. Unoccupied sites were estimated by the addition of 3H-B (3.5 x 10-8M) to aliquots of the homogenate.

In the stressed rat, the pattern of regional binding differed dramatically from that previously reported in the ADX rat: the pituitary, not the hippocampus, showed the highest binding expressed as f moles/mg protein: pituitary 693, preoptic 362, septum 289, hypothalamus 239, amygdala 154, cortex (CX) 168, hippocampus (HC) 186, and midbrain (MB) 134. Mean plasma B value was 58 ug%, which resulted in a brain B level of 7.5 p moles/mg protein. The number of total binding sites (endogenous + 3H-B) in the 3 regions studied (CX, HC and MB) showed little regional difference. ADX animals had a mean plasma B value of <1 ug%. Total binding sites in ADX rats were higher than in intact rats, with HC showing the greatest number of sites as expected. The results 1) support the concept of the pituitary as the primary site of glucocorticoid feedback control, 2) indicate that the ADX rat is not an adequate model for physiological studies of brain-glucocorticoid interaction, 3) suggest that brain regions other than the HC need closer investigation in stress studies. (Supported in part by NIMH training grant 07417 to B.T.)

1483 DISCRETE BINDING OF [^{3}H]EPINEPHRINE TO CALF BRAIN α- AND β-NORADRENERGIC RECEPTORS. David C. U'Prichard, David A. Greenberg and Solomon H. Snyder. Depts. Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD. 21205.

In the presence of 1 mM pyrocatechol, the catecholamine neurohormone [^{3}H]epinephrine displays saturable, high affinity binding to α-noradrenergic receptors in most gray matter regions of the calf brain. Specific binding was highest in the frontal cortex, with other cortical areas, hippocampus, amygdala and hypothalamus having 60-80% of frontal cortex values. The thalamus and caudate nucleus had appreciable α-receptor [^{3}H]epinephrine binding, about 50% of frontal cortex, whereas midbrain and brainstem regions were much lower. White matter areas possessed very low levels of specific binding. At 37°C, the K_D of [^{3}H]epinephrine was 18 nM, whereas after incubation at 25°C the affinity of [^{3}H]epinephrine was increased to a K_D of 5 nM, without alteration of the B_{max}. At all temperatures and in all regions, specific binding possessed α-receptor SAR, with epinephrine a 2-3 times more potent inhibitor than norepinephrine, and 500 times more potent than isoproterenol. Phentolamine, clonidine and ergot alkaloids had K_i values in the 1-10 nM range. High levels of α-receptor binding were found in calf cerebellum, about 60% of that in frontal cortex, and when specific α-receptor binding in that tissue was blocked with phentolamine, a significant portion of the residual binding, displaceable by propranolol, showed characteristics of binding to noradrenergic receptors of the β_2 type. Specific binding of [^{3}H]epinephrine to β-receptors was saturable, with a K_D of 30 nM at 25°C. Isoproterenol was a very potent inhibitor of β-receptor binding, with epinephrine and norepinephrine 10 and 100 times weaker respectively. Propranolol and alprenolol were as potent in competing for binding as isoproterenol, but other β-antagonists were substantially less potent. Stereospecificity of catecholamines and antagonists at β-receptor binding sites ranged from 20 to 100 fold in favor of the (-)-isomers. Binding of [^{3}H]epinephrine to β-receptors was not observed in any other calf brain region. Numerous previous attempts to demonstrate specific binding of endogenous catecholamines to α-, and especially β-receptors in various tissues have been unsuccessful. The discrete binding of [^{3}H]epinephrine to both α- and β-receptors in calf cerebellum reported here provides a suitable model for examining differential effects of ions, temperature-induced receptor interconvertibility and cellular localization of cerebellar α- and β- sites. These results will be discussed. (Supported by USPHS grants MH-18501, MH-33128, MH-05105 and DA-05052.)

1484 VOLTAGE DEPENDENT MODULATION OF β-ADRENERGIC RECEPTOR MEMBRANE BINDING IN RAT BRAIN SLICES. H. R. Wagner* and J. N. Davis (SPON: E. W. Busse). VA Hospital and Duke University, Durham, NC 27705.

Incubation of rat cerebral cortex slices with the β-adrenergic agonist, isoproterenol (ISO), leads to a dose-dependent decrease in β-adrenergic receptor (βAR) binding sites as measured with the βAR ligand, [^{3}H] dihydroalprenolol (DHA). We previously suggested that the ISO-induced decrease in DHA binding sites represents βAR desensitization in brain slices. During the course of those studies, we observed that factors which depolarize brain slices also produce a rapid recovery of lost DHA binding sites. To systemically investigate the effects of membrane voltage on the ISO-induced loss of DHA binding sites, we exposed slices of rat brain cerebral cortex to 10 μM ISO for 30 minutes. Various depolarizing agents were added for the final 10 minutes of this period. At the end of the incubation, membranes were prepared from slices, extensively washed to remove any traces of ISO, and βAR binding was measured by incubation with 10 nM [^{3}H] DHA followed by rapid filtration. ISO-incubated slices had 70% of the DHA binding found in membranes from control slices incubated in buffer alone. Exposure of ISO-treated slices to high K^+ (50 mM), grayanotoxin (10 μM), or batrachotoxin (10 μM) in the continued presence of ISO restored DHA binding to 96%, 95%, and 100% of control. Exposure of slices to grayanotoxin (10 μM) did not restore DHA binding in a Na^+-free (choline) buffer or when the slices were pretreated with tetrodotoxin (10 μM). Pretreatment of slices with EDTA and removal of Ca^{++} from the incubation did not prevent grayanotoxin reversal of DHA binding. These data support our previous proposal that the ISO-induced loss of DHA binding sites represents βAR desensitization. Our results suggest that membrane depolarization resensitizes βARs through a voltage-dependent movement of Na^+ ions and that βAR desensitization like nicotinic cholinergic and α-adrenergic receptor desensitization is regulated in part by membrane voltage.

1485 DIFFERENTIAL DEVELOPMENT OF β-ADRENERGIC AND DOPAMINE RECEPTOR-CYCLASE SYSTEMS IN CULTURED AGGREGATES OF BRAIN CELLS FROM THE PRENATAL RAT. Kenneth G. Walton, Ronald Majocha,*G. Robert DeLong* and Ross J. Baldessarini. Mailman Research Center, McLean Hospital, Belmont, MA 02178 and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Working independently, DeLong (Dev. Biol. 22:563, 1970) and Seeds (PNAS 68:1858, 1971) developed techniques for maintaining rodent brain cells as spherical reaggregates of dissociated cells by growing the cells on a rotary shaker. Brain-cell aggregates grown in this manner show changes in culture which resemble the changes known to occur in intact developing brain. Examples include biochemical differentiation, specific cell sortings and synapse formation. We have begun studies on receptor-adenylate cyclase systems in these aggregates with the hope of using this preparation to increase our understanding of the characteristics and plasticity of such receptor-cyclase systems as well as their role in synaptic transmission and possible role in brain development. Using aggregates prepared from cells of whole brains of 17-day prenatal rats we have found separate stimulations of cyclic AMP levels mediated by β-adrenergic and dopamine receptors in intact aggregates. A similar degree of stimulation (about 2-fold) was obtained through each of these receptor systems after one day in culture, with a gradual increase in stimulation over the next 9 days. Between 10 and 18 days in culture, the β-adrenergic-receptor-mediated stimulation increased dramatically to 7-8 fold whereas the dopamine-receptor-mediated stimulation increased only to 4-5 fold. Both the magnitude and the time course of the changes in β-adrenergic receptor-cyclase resemble those known to occur in intact rat brain during early development. For example, Harden et al. (Brain Res. 125:99, 1977) found a dramatic increase in β-adrenergic receptor during the second postnatal week in cerebral cortex. These results suggest that brain-cell aggregates will prove valuable for studying receptor-cyclase systems in intact cells and possibly also for studying aspects of brain differentiation and development relating to the cyclic nucleotides.

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1486 OVERLAP OF DOPAMINE- AND SEROTONIN-DISPLACEABLE LSD BINDING IN COW CAUDATE. Patricia M. Whitaker* and P. Seeman (Spon: J. Warsh). Pharmacology Department, University of Toronto, Toronto, Canada.

Lysergic acid diethylamide (LSD) has been reported to exert its effects either through actions in the dopaminergic (Pieri et al., Nature 252: 586, 1974) or in the serotoninergic system (Bennett and Aghajanian, Life Sci. 15: p. 1935, 1974). Our work suggests that, at least in some circumstances, these two neurosystems may share a common set of receptors for LSD.

The binding of ^{3}H-LSD was done on homogenates of calf caudate nuclei (Seeman et al., P.N.A.S. 72: 767, 1975). ^{3}H-LSD binding was also measured in the presence of excess (1 µM) dopamine, serotonin or epinephrine, or in a combination of these.

LSD was found, by Scatchard analysis, to occupy sites equivalent to 825 fmoles/mg, with a K_D for these sites of 8.4 nM. The number of ^{3}H-LSD sites dropped to 615 fmoles/mg in the presence of excess dopamine (DA), and to 525 fmoles/mg in the presence of excess serotonin (5HT). However, when both neurotransmitters (DA + 5HT) were present in excess, the number of ^{3}H-LSD sites was still only reduced to 565 fmoles/mg.

If the receptor sites occupied by these two substances were completely separate, one would expect a cumulative decrease in ^{3}H-LSD binding, i.e. to about 315 fmoles/mg. The fact that the decrease was not this great suggests that dopamine and serotonin are displacing ^{3}H-LSD from similar or closely related sites, with the possible exception of approximately 50-90 fmoles/mg which appear to be purely serotoninergic sites.

Epinephrine had no effect in reducing binding of ^{3}H-LSD, suggesting that these receptors are not involved in LSD binding. As would be expected, therefore, in the presence of excess epinephrine plus excess serotonin or dopamine, the resultant decrease in binding was identical to that seen in the presence of serotonin or dopamine alone.

This work suggests that at least a considerable proportion (over 75%) of the serotonin and dopamine receptors overlap with each other, insofar as they contribute to the binding of ^{3}H-LSD. (Supported by Ontario Mental Health Foundation and the Medical Research Council of Canada).

1487 INNERVATION OF SOME DEEP TISSUES IN THE THORACOLUMBAR REGION OF MAMMALS. David G. Whitlock, Priscilla A. Ledbury*, Lanay J. Land, and Ralph A. W. Lehman*. Dept. of Anat., Univ. of Colo. Med. Sch., Denver, CO 80262.

Few studies have been made of the innervation of deep tissues; the only notable exception being investigations of the innervation of muscle spindles and Golgi tendon organs. In our experiments autoradiography has been used to examine some deep tissue pathways and their terminations in the thoracolumbar region of mammals. Concentrated L-3,4(n)^{3}H-proline (0.2-1.2mCi/µl) was hydraulically injected into either a mid-thoracic dorsal root ganglion or a celiac collateral ganglion of anesthetized monkeys or cats. After a post-injection survival period of 7-14 days the animals were reanesthetized, transcardially perfused with Bouin's solution, and all tissues of interest were removed, embedded in paraffin, serially sectioned at 3-5 microns and prepared for autoradiography.

Following such an injection into a T-8 dorsal root ganglion, radioactivity was observed in two types of receptor-like structures in the thoracic wall. The first of these structures occurred in clusters adjacent to the intercostal muscles. Each element appeared to be innervated by a single labelled axon. They apparently did not contain either intrafusal fibers or evidence of secondary innervation. Usually they were tightly adherent on one side to the fleshy belly of the muscle and were never associated with the tendons. The second type of receptor-like structures we observed consisted of small nodules attached to the walls of the intercostal veins. A single labelled axon could be traced into each nodule where it terminated, as indicated by a spray of silver grains overlying the nodular structure and directed towards the vein lumen. We conclude that both of these structures must be afferent in character for their innervation could only have come from the labelled cell bodies of the injected mid-thoracic ganglion.

Injection of radioactive proline into a celiac collateral ganglion gave rise to a small number of labelled axons distal to the ganglion. Some of these axons could be traced through the connective tissue sheath surrounding the adrenal and along the connective tissue septa within the adrenal cortex into the adrenal medulla. A single labelled axon could be traced within the medulla to its apparent termination on the wall of a medium sized artery. This termination, we believe, is part of a visceral motor system. It may modulate the activity of the adrenal medulla through control of its blood supply.

(Supported, in part, by USPHS Grant NS-08453.)

SLEEP

1488 TEMPORAL CHARACTERISTICS OF SLEEP IN MICE. Helen A. Baghdoyan* and Matej Stepita-Klauco (SPON: V. L. Friedrich). Dept. of Biobehavioral Sciences, U.Conn., Storrs, Ct. 06268.

Sleep in mice (C57BL/6) was studied by means of chronic (3-4 weeks) polygraphic recording. The method employed time compressed (TC) EEG and head movement signals which were continuously recorded on chart paper (3 mm/min.). Portions of the raw EEG were simultaneously recorded on magnetic tape for subsequent spectral analysis. The pen record of the TC signal was assessed visually. Three states of vigilance were recognized: waking, REM sleep and NREM sleep. The reliability of the TC signal for assessing the behavioral states of the animal was tested by comparing the coincidence of states obtained by a)scoring the pen recorded TC signal and b)behavioral observation of the animal with concurrent examination of the raw EEG displayed on an oscilloscope. Scored and observed states were compared for 10 second epochs. Their matching was expressed as a percentage of the total duration of each state over the time interval of the experiment. Comparisons were made for scored (TABLE I) and observed (TABLE II) states taken as the reference and expressed in percents of the duration of the state listed in the left column ± S.E.M (N=5).

TABLE I	SCORED	O B S E R V E D		
		WAKE	NREM	REM
	WAKE	91.19 ± 3.77	6.56 ± 2.84	2.25 ± 1.33
	NREM	11.93 ± 2.52	84.50 ± 2.70	3.56 ± 1.46
	REM	16.30 ± 4.66	18.75 ± 2.13	64.95 ± 6.32

TABLE II	OBSERVED	S C O R E D		
		WAKE	NREM	REM
	WAKE	73.96 ± 4.27	21.78 ± 4.90	4.26 ± 1.26
	NREM	2.91 ± 0.98	93.12 ± 1.05	3.08 ± 0.36
	REM	6.78 ± 4.12	25.42 ±10.32	67.80 ± 6.95

The time spent in each state per day showed small variations. During a 24 hour period 613.8 ± 13.9 min.(42.7%) were spent in waking and 824.3 ± 13.9 min.(57.3%) in sleep. The sleeping time consisted of 739.3 ± 12.7 min.(51.4%) of NREM and 85.0 ± 3.4 min. (5.9%) of REM sleep. Spectral analysis revealed a dominant frequency in the range between 6-9 Hz. for both waking and REM sleep. This activity was most prominent during REM sleep, whereas in waking additional activity at lower frequencies was present. The mean peak frequencies for waking (7.41 ± .25 Hz., N=14) and REM sleep (8.00 ± .12 Hz., N=13) were not significantly different. The mean peak frequency for NREM sleep was 2.34 ± .21 Hz. (N=20). No cortical spindle activity during NREM sleep was observed.

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1489 ACTIVE SLEEP REVERSES RESPIRATORY FAILURE IN HYPOXIC KITTENS. T. L. Baker* and D. J. McGinty. V. A. Hospital, Sepulveda, CA 91343 and University of California, Los Angeles, CA 90024.

We assessed the effects of chronic intermittent hypoxia upon sleep patterns, respiration, and cardiac function in 10, 20 and 40 day old kittens. Forty kittens at each age were isolated in Plexiglas chambers at 21% O_2 (controls), 10% O_2 or 7% O_2 for four 2-hour 'conditioning' sessions daily for three or eight days. Kittens were surgically implanted with chronic electrodes for measurement of EEG, EOG, neck EMG, EKG and with a nasal thermistor to measure respiratory air flow. Physiological parameters and a time code were recorded polygraphically and on magnetic tape. Each minute of polygraphic data was scored as waking (W), active sleep (AS), quiet sleep (QS) or transitional-indeterminate (T). Respiratory rate and variability, and heart rate and beat-to-beat variability were derived by computer analysis.

At all ages hypoxia reduced AS and augmented QS and T. Individual epochs of AS were shorter and less frequent in hypoxic subjects compared to controls. Similarly, frequency and length of QS epochs was increased. During experimental hypoxemia, some kittens repeatedly exhibited a respiratory 'failure' syndrome during QS, T or W. These episodes consisted of slow apneustic-type breathing accompanied by bradycardia and other EKG irregularities. Active sleep onset consistently stimulated respiration and heart rate and rectified irregularity in these functions, thereby reversing hypoxemic 'crises.' Hypoxic kittens that exhibited most severe cardiopulmonary failure and died showed more extensive suppression of AS than other subjects in their respective age groups.

We hypothesize that neural control of respiration during AS is stable and resistant to hypoxemia. Active sleep may serve a protective function during hypoxemic stress, especially during development. This theory is consistent with a recent report that sleep-related respiratory depression in infants (Ondine's Curse) is reversed during AS. Hypoxemia and sleep-related respiratory failure have also been implicated in the Sudden Infant Death Syndrome (SIDS). The natural predominance and tenacity of active sleep in the newborn may explain the paradoxically rare occurrence of SIDS in the first month of life.

1490 CHARACTERIZATION OF SYNAPTIC POTENTIALS INDUCED BY RETICULAR FORMATION STIMULATION ON TRIGEMINAL MOTONEURONS DURING SLEEP AND WAKEFULNESS. Scott H. Chandler*, Yoshio Nakamura*, and Michael H. Chase (SPON: R.E. Hall). Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

We have previously reported that excitation of the ponto-mesencephalic reticular formation induces, in trigeminal jaw-closer motoneurons, depolarization during wakefulness and quiet sleep, and hyperpolarization during active sleep, i.e., state-dependent response-reversal. We have investigated the nature of these synaptic potentials in 4 cats which were unanesthetized and freely-moving (with the exception of head restraint). They were implanted under nembutal anesthesia with standard EEG, EOG and EMG recording electrodes. Jeweler screws were fixed in the mandibular canal to stimulate the inferior dental nerve. Bipolar stimulating electrodes were implanted in the mesencephalic Vth nucleus and the nucleus reticularis pontis oralis. 3 M KCl or 2 M K-citrate micropipettes were used (with tip resistances of 8-15 megohms) for intracellular recording from trigeminal motoneurons. A short train of conditioning pulses (1-3 pulses; 500 cps; .3 msec) was delivered to the nucleus reticularis pontis oralis at a rate of 1/sec. The amplitude of the control depolarizing potential increased and decreased, respectively, when hyperpolarizing and depolarizing currents were passed through the recording electrode. Therefore the depolarizing potential is membrane potential dependent and can be considered to be an EPSP. The hyperpolarization induced by reticular stimulation during active sleep was compared with the somatic IPSP evoked by inferior dental nerve stimulation following the 1)injection of chloride into the cell and 2)alteration of the membrane potential by passage of current from a K-citrate electrode. The hyperpolarization to reticular stimulation during active sleep was much less sensitive to injected current from a K-citrate microelectrode than the somatic IPSP induced by inferior dental nerve stimulation. When chloride was injected into the cell the IPSP to inferior dental nerve stimulation was reversed in polarity while the hyperpolarization to reticular stimulation was only reduced in amplitude. Therefore the synaptic basis for the reticular response-reversal can be explained as follows: During wakefulness and quiet sleep a short latency (3-5 msec) postsynaptic depolarizing potential occurs. During active sleep the depolarization is reduced in amplitude and a longer latency and duration hyperpolarization emerges which is also postsynaptic. However, on the basis of its reduced sensitivity to chloride injection we conclude that this area of the reticular formation exerts its inhibitory effect through a system(s) which has its inhibitory input located predominately onto the dendrites of the motoneurons. Supported by USPHS 09999.

1491 TRIGEMINAL MOTONEURON SPIKE POTENTIAL ACTIVITY DURING SLEEP AND WAKEFULNESS: CORRELATIONS WITH NECK EMG DISCHARGE. Michael H. Chase, Scott H. Chandler*, Benjamin Chang*, and Yoshio Nakamura*. Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

The modulation of electromyographic (EMG) activity during sleep and wakefulness has been extensively studied for the past twenty years. These studies have described both phasic and tonic variations in the level of EMG activity as a correlate of the animal's state. We have monitored the discharge and membrane potential activity of trigeminal motoneurons and correlated this activity with variations in neck EMG, which in the cat, is typically used as general indicator of state-dependent variations in motor discharge. In 4 chronic cats that were undrugged, intact, normally respiring and freely-moving (with the exception of head restraint), micropipettes (filled with 2M K citrate; tip resistances of 8-15 megohms) were inserted into 20 electrophysiologically identified trigeminal motoneurons. The activity of these (jaw-closer) motoneurons was correlated with the EMG of the neck musculature during sleep and wakefulness. High motoneuron discharge rates were observed during wakefulness, when EMG activity was greatest. In general, bursts of EMG activity were accompanied by a decrease in membrane potential and an increase in spike potentials. Reduction in EMG activity was accompanied by an increase in membrane polarization and decreased spike activity. During quiet sleep the rate of spike activity was also correlated with tonic and phasic EMG discharge in the same manner as during wakefulness. During active sleep the membrane potential increased by as much as 10 mV. This increase was accompanied by a decrease in neck EMG activity and trigeminal motoneuron discharge. However, in conjunction with bursts of neck EMG activity and phasic muscular twitches, the level of motoneuron polarization decreased phasically and spike activity was initiated. This correlation was typically present, but not absolute. These findings indicate that the control of motor activity in the trigeminal system reflects the phasic and tonic modulation of spinal cord motor activity and exhibits state dependency of both membrane potential and spike discharge. The basis for this control at a synaptic level can now be studied by utilizing the intracellular technique to determine the causal processes which control the level of polarization and discharge of motoneurons during sleep and wakefulness. Supported by USPHS NS 09999.

1492 RESPIRATORY RESPONSES TO AIRWAY OBSTRUCTION DURING SLEEP AND WAKING IN THE DOG. Susan DeMesquita* and Eugene Aserinsky. Dept. Physiol., Sch. Med., Marshall Univ., Huntington, WV 25701.

Nine adult dogs with in-dwelling tracheostomy tubes were monitored for respiratory parameters during 8-hr sleep trials with the tubes patent for 4 hrs, and partially plugged for 4 hrs -- the diameter of the orifice being reduced by ca. half to an end diameter of 2 to 4 mm. The partially occluded airway caused labored breathing in the waking state but did not prevent the onset of sleep. The purpose of the experiment was to determine how physiological responses to stress might differ during different states of consciousness.

Records were scored min by min for each of three states (Waking, REM Sleep and Non-REM Sleep) which had been identified by EEG, neck EMG and EOG. Airway obstruction had no signif. effect on total sleep time, REM % and REM Pd. duration. With the airway patent, the mean± S.D. values for TST, REM % and REM Pd. duration were 136±37 min, 17.1±6.2% and 5.7±1.4 min, respectively; the corresponding values while the tubes were plugged were: 146±35 min, 17.7±5.7% and 5.5±1.0 min. However, preliminary analysis of eye mvt data suggests a marked increase in REM density (eye mvts/min) during airway obstruction.

Mean respiratory values obtained while the tracheal tubes were unobstructed and then obstructed are tabulated below:

	WAKE		Non-REM SLEEP		REM SLEEP	
	Unobst.	Obst.	Unobst.	Obst.	Unobst.	Obst.
Breaths/min	80	52	19	16	18	13
V (l/min)	7.4	4.8	3.1	2.3	3.3	2.2
Insp. (sec)	0.5	0.7	1.1	1.5	1.2	1.6
Exp. (sec)	0.5	1.1	0.8	2.1	0.8	2.1
$P_A CO_2$ %	4.4	5.2	5.4	6.1	5.3	5.9

A direct effect of increased airway resistance was a signif. ($p<.01$) increase of ca. 35% for inspiratory duration and over 100% for expiratory duration in all three states of consciousness. This led to signif. decreases in respiratory rate and min volume in the three states but the mean tidal volumes did not change. Compensation was not complete in view of the signif. rise of alveolar CO_2. The relatively low CO_2 values in Waking may have been due to hyperventilation accompanying awareness of the experimental situation.

While the initial or control values in Waking were different from the sleep states, the respiratory responses to the stress were proportionately the same in all three states. These results do not support the notion that REM sleep is a time of especial vulnerability to stress.

1493 A NONLINEAR CLASSIFIER FOR AUTOMATED SCORING OF SLEEP STAGES IN CATS: DEVELOPMENT AND USE IN EVALUATING DRUG EFFECTS. A. T. Dren*, W. J. Giardina, C. H. Nute*, J. P. Hansen*, W. G. Jochimsen*, A. N. Mucciardi* and R. Shankar*. Abbott Lab., No. Chicago, IL 60064 and Adaptronics, Inc., McLean, VA 22101.

An adaptive learning network sleep stage classifier (ALNSSC) was developed for automatic scoring of sleep stages in the cat. Using heuristic classification algorithms, ALNSSC was trained on a data base of over 4,000 manually scored epochs obtained from 5 cats implanted with EEG, EMG and EOG electrodes. Calibrated electrophysiological signals were amplified with a Grass Model-78 polygraph. For each animal, the analog output of the polygraph was collected and digitalized on a PDP 11/50 computer in consecutive 20 second time blocks (17 seconds of data) for at least 8 continuous hours. These data were transformed using a Fast Fourier algorithm to provide a total of 35 parameters for each epoch: power spectra of the 3 EEG leads in 2 Hz band widths and total power readings of the EMG and EOG leads. For training the classifier, the epochs were manually scored according to a 4 stage classification: awake, spindle sleep, slow wave sleep and rapid eye movement sleep (REM). The mean agreement of ALNSSC computed sleep stage determinations to manually derived scores was 83% with a range of 78% to 93%. Data base considerations and characteristics of the sleep stage prototypes will be available for discussion. In its present configuration, the PDP 11/50 parameterizes the polygraph signals on-line and compares the digitalized data to sleep stage prototypes in the classifier at the end of each experimental session. Data from up to 4 separate experimental chambers can be processed simultaneously for a maximum of 15 continuous hours. Epochs have been lengthened to 30 seconds and the ALNSSC classifies each into one of the 4 stages. Auxiliary programs calculate numerical values for the amount of time in each stage and the relative percentage of each sleep stage, and provide temporal graphic displays of sequential changes in sleep patterns. The latency to sleep onset and to the first REM period are also obtained from the computer output. The application of the ALNSSC to pharmacologically altered sleep patterns will be illustrated with data obtained with psychostimulant and CNS depressant drugs.

1494 MULTIPLE UNIT ACTIVITY DURING TONIC AND PHASIC REM SLEEP: MODIFICATIONS BY PROTEIN SYNTHESISINHIBITORS. René Drucker-Colín, José Bernal*, Francisco Díaz-Mitoma* and Jorge Zamora*. Dept. Biol. Exptl. Inst. Biol. Univ. Nac. Autónoma de México. México, D. F.

Rapid Eye Movement (REM) sleep can be divided into periods with phasic activity (REMp) and periods with tonic activity (REMt). The object of this study is to determine whether inhibitors of protein synthesis modifies such periods. Twenty cats were stereotaxically implanted with microelectrodes (60 µm) in the hipocampus (H), midbrain reticular formation (MRF) and in some cases in the amygdala and cerebellum. The animals were additionally implanted with screw electrodes in the cortex, and external canthus of the eye, a bipolar electrode in the lateral geniculate (LGB) and electrodes in the muscles of the neck. Multiple unit activity (MUA) and the sleep-wake cycle of each cat was recorded several times for periods varying between 5-12 hours following the administration of saline, anisomycin (5-10 mg/kg), and cloramphenicol (50-100 mg/kg). Some cats were also recorded for 2 hours following the administration of amphetamine (10 mg/kg). The results of these experiments showed during control recordings that REMf and REMt alternated in a cyclic fashion, but only when the duration of the REM sleep periods was longer than 6 minutes. It was also found that in 31 of 83 long REM periods during REMf the frequency/sec of MUA in H was low (4.50 ± 0.36) and high in MRF (11.25 ± 0.76) while during REMt MUA frequency in H was high (10.22 ± 0.52) and low in MRF (4.21 ± 0.30). The same inverse phenomenon was observed following amphetamine, during presence or absence of eye movements. Following the administration of anisomycin and cloramphenicol the frequency of REM sleep decreased, and there was augmentation of REMt, while PGO spikes decreased. Also it could be observed that there were periods of REM without atonia. It is suggested that the inverse and oscillatory changes in excitability and their modifications produced by protein synthesis inhibitors may provide support for the idea that REM sleep represents a period during which the brain "reprogramms" itself.

1495 COMPARISON OF REVERSIBLE CRYOGENIC BLOCKADE TO IRREVERSIBLE LESIONS IN THE PONTINE TEGMENTUM OF CATS. Loyd L. Glenn, Wesley B. Leung*, and William C. Dement. Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305.

Bilateral lesions of the dorsolateral pontine tegmentum can produce hallucinatory behavior in animals during REM sleep. This is presumably due to an abolition or counteracting of the neural mechanisms underlying the muscular atonia seen during REM sleep. We attempted to reversibly produce REM sleep without atonia by cryogenic blockade of this region. Three cats were implanted for cortical, ocular, and PGO wave recordings, in addition to the bilateral placement of cryodes in the dorsolateral pontine tegmentum. The cryodes consisted of two concentric, uninsulated, stainless steel cylinders (22 gauge O.D.) cooled at the tips by the flow of cold, pressurized helium (-70°C. @ 200-600 PSI). Cats were deprived of REM sleep for 24 hours before experimentation on a slowly moving treadmill, then atraumatically restrained within a sound-attenuating booth. Pairs of subdermal electrodes were inserted into four muscles for the recording of general electromyographic activity. Cryode coolings occurred on alternate REM periods in 30 second pulses. Rather than increasing postural tonus during REM sleep, unilateral and bilateral cryogenic blockade always caused a strong arousal to wakefulness. To investigate the reason for the difference between effects obtained by cryodes from that of electrocoagulation, irreversible electrolytic or cryogenic lesions were made through the cryodes. These lesions, made under restrained, unanaesthetized conditions, produced an immediate long-lasting arousal for 6 to 30 hours, followed by the gradual reappearance of NREM sleep. The arousing effect of temporary cryogenic blockade is probably related to the initial longlasting wakefulness seen after a lesion, but the chronic effects of a lesion do not occur in such short duration blockades. The proper placement of the cryode tips is indicated in each cat by (1) the transient appearance of REM sleep without complete atonia after the cryode placement alone, (2) the total suppression of REM sleep by irreversible cryogenic and electrolytic lesions made through the cryodes, and (3) the histologically verified placement of cryode tips in regions established by others to produce REM sleep without atonia when lesioned. The initial wakefulness seen after irreversible lesions in the dorsolateral pontine tegmentum are similar to the effects of temporary blockade of this region. However, this study and the work of others indicates that the chronic effects of permanent lesions do not predict the effect of a temporary cryogenic blockade in the same region.

496 THE EFFECT OF REM SLEEP DEPRIVATION ON STIMULUS-BOUND EATING THRESHOLDS. R. Halperin*, J. Halperin*, F. Villegas*, S. Daren*, S. Steiner*, and S. Ellman. Dept. Psychol., CCNY, New York, N. Y. 10031.

REM sleep deprivation (RD) lowers thresholds for lateral hypothalamic (LH) intracranial self-stimulation (ICSS). D-amphetamine also lowers thresholds for LH ICSS. D-amphetamine raises thresholds for stimulus bound eating (SBE) electrically elicited from LH ICSS sites. This study investigated the effect of RD on SBE thresholds.

Six rats, maintained on an 1-12, d-12 lighting schedule were stereotaxically implanted with a bipolar electrode aimed at the LH, EEG screws and an EMG electrode. SBE thresholds, using 60 Hz. stimulation in a modified method of limits, were obtained either three hours into the dark period or 10 1/2 hours into the light period. After determination of stable SBE thresholds, the experimental paradigm consisted of two days of adaptation (A), five days of baseline (BL), five days of RD and five days of recovery (R). Animals were housed in sawdust during A, BL and R conditions. RD was achieved by maintaining animals on a small platform surrounded by water. Each animal was also tested in a large platform (LP) control condition; conditions were counterbalanced across animals.

All platform conditions significantly increased SBE thresholds over BL levels, however during RD, thresholds were significantly higher than during LP. All thresholds returned to BL levels during R. Increases in SBE thresholds occurred during both lighting conditions.

The effects of RD on both ICSS and SBE parallel the effects that result from administration of d-amphetamine. We interpret these results as implicating catecholamines as a common mechanism of action for RD and d-amphetamine.

1497 DECREASED CARDIORESPIRATORY COUPLING IN INFANTS AT RISK FOR THE SUDDEN INFANT DEATH SYNDROME. R.M. Harper, D.O.Walter, B. Leake, T. Hoppenbrowers*, M.B. Sterman, and J. Hodgman*. Sepulveda VAH, Dept. of Anatomy, UCLA and County USC Medical Center, Los Angeles, CA.

Seven normal infants and seven infants at risk for Sudden Infant Death Syndrome (SIDS) were instrumented with ECG, expired CO_2, EEG, EOG and somatic activity electrodes and recorded for 12 hr periods at 1 week and 1,2,3,4 and 6 mos. of age. The degree of cardiac variability that was due to respiration was assessed by calculating the coherence between cardiac rate and respiratory activity at the median respiratory rate for each minute. Coherence is analogous to a squared coefficient of correlation between respiratory activity and heart variability.

Successive minutes of each 12 hr recording were classified as quiet sleep (QS), active sleep, waking or indeterminate states by trained observers. Coherence values for each minute of the 12 hr record were sorted by sleep state, and average values for each sleep period were obtained for the entire recording. Effects of age, state and risk were assessed using analysis of variance and Duncan multiple range test procedures. In both normal and risk infants, cardiorespiratory coupling was higher in QS than in active sleep or waking. Coherence values in QS also dropped from the newborn period to 1 month of age, and then rose to the 6 month age period.

Coherence was lower for infants at risk for Sudden Infant Death than for normal infants in QS and in waking over all ages. This failure of regulation of cardiac rate variability by respiratory effort in the SIDS risk group may indicate a dysfunction of receptors sensitive to intrathoracic pressure change, or an abnormality in autonomic efferent activity to the heart.

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1498 THE EFFECT OF METHYSERGIDE ON 5-HYDROXYINDOLE ACETIC AND HOMOVANILLIC ACIDS IN THE CISTERNAL CEREBROSPINAL FLUID OF CATS. H. Jackman and M. Radulovacki. Dept. Pharmacol., Univ. Ill. Med. Ctr., Chicago, IL 60612.

We have reported that methysergide maleate (ME), a competitive blocker of 5-hydroxytryptamine (5-HT) receptors, delays sleep onset and disrupts sleep patterns in cats when administered at a dose of 0.5 mg/kg. This disturbance of sleep in cats caused by ME lends support to the suggested requirement of a properly functioning 5-HT system involved in the mechanisms of endogenous sleep. Since 5-HT and dopamine (DA) have been implicated in the mediation of sleep and wakefulness, respectfully, we wanted to measure the metabolism of these monoamines by determining the concentrations of their major metabolites 5-hydroxyindole acetic acid (5-HIAA) and homovanillic acid (HVA) in cerebrospinal fluid (CSF) after 0.5 mg/kg ME. Five cats were implanted with cannulas to the cisterna magna (Radulovacki, 1974). Control injections of Ringer's solution, ip, were administered for three days and 1-ml samples of CSF were collected at two-hour intervals starting at 9 A.M. and ending at 5 P.M. On the fourth day, 0.5 mg/kg ME, ip, was administered at 10 A.M. and CSF samples were collected as in controls. The amine metabolites were assayed fluorometrically by the methods of Korf and Valkenburgh-Sikkema (1969) and Sato (1965). Our results are as follows:

	5-HIAA ng/ml CSF±S.E.		HVA ng/ml CSF±S.E.	
Sample	Control	0.5 mg/kg ME	Control	0.5 mg/kg ME
9:00	97 ± 14	122 ± 23	75 ± 10	86 ± 21
11:00	*100 ± 14*	*114 ± 25*	*108 ± 18*	*150 ± 36*
1:00	104 ± 13	127 ± 22	99 ± 10	116 ± 19
3:00	94 ± 12	108 ± 14	99 ± 12	96 ± 27
5:00	107 ± 12	129 ± 25	89 ± 14	109 ± 20

Our data show no significant differences in the 5-HIAA or the HVA concentrations between the control and ME-treated cats. Therefore, suppression of sleep by ME is accomplished without affecting the turnover of central 5-HT and DA. We have interpreted the effects of ME on sleep as reflecting the blockade of central 5-HT receptors. In light of this and our present findings, increased wakefulness could be mediated by a normally functioning DA system while the 5-HT system shows no compensatory mechanism on receptor blockade with ME.

1499 AN EXPERIMENTAL STUDY OF HYPOTHALAMIC AREAS AND CONNECTIONS RELATED TO SLEEP. F. Cleveland Kinney* (SPON: Elizabeth C. Crosby). Dept. Anat., Sch. Med. and Dent., Univ. Alabama in Birmingham, Birmingham, AL 35294

It is well documented that bilateral lesions located in caudal hypothalamic areas, and/or lesions at the midbrain diencephalic junction, result in narcolepsy and catalepsy in cat, monkey and man. However, the specific areas of the hypothalamus concerned have not been clearly delineated. In this study, bilateral, electrolytic stereotaxic lesions of the hypothalamus involving primarily the ventromedial nucleus and the posterior hypothalamic area resulted in prolonged sleep, or narcolepsy, for eight days and continual hypothermia in one cat and sleep for three days in another cat. During wakeful periods, circling behavior was observed in those animals in which the lesions extended into the overlying ventral thalamus. In a third cat, bilateral electrolytic stereotaxic lesions placed slightly caudal to the posterior hypothalamic area and which incidentally involved the basilar artery resulted in coma and decerebrate rigidity. Coma, resulting from this lesion of the mesencephalic diencephalic junction differed from those responses from the above mentioned hypothalamic lesions. Also, this animal was unresponsive to pain and lacked a corneal reflex following surgery. Behavioral changes following the placement of bilateral lesions slightly rostral to and overlapping the posterior hypothalamus were observed in one cat. This cat was generally unresponsive to its environment; it was slow to move and appeared to be somewhat obtunded. Another cat underwent surgery during which the fornix was partially interrupted bilaterally. This partial bilateral interruption of the fornix failed to produce sleep. The results from the stereotaxic lesions placed in the hypothalamus were contrasted with partial and complete lesions of the fornix and the hippocampal areas at different levels.

The Nauta technique for impregnating degenerated axons with silver was used to follow the degenerated pathways. Degenerated axons were traced to their terminations and their connections will be discussed.

1500 CIRCADIAN DISRUPTIONS OF SLEEP IN MICE: CAN THE SLEEP RHYTHM DISSOCIATE FROM THE ACTIVITY RHYTHM? Merrill M. Mitler*, Fred A. Vincent*, and William C. Dement. Sleep Disorders Center, Stanford U. Sch. Med., Stanford, CA 94305

Chronically implanted C57BL/10J (N=11) and BALB/cJ (N=2) mice were continuously monitored by running-wheel sensor for activity and by 96-hour polygraphic recordings for Wakefulness, NREM, and REM sleep. Forty-nine recordings from a two-year period were used to describe the temporal distribution of activity and sleep in these M. musculus strains. Daily (under LD) and circadian (under DD and LL) peaks in NREM sleep and REM sleep tended to coincide temporally. Daily and circadian peaks in Wakefulness and Wheel running also tended to coincide temporally. The segregation between NREM and REM sleep on the one hand and Wakefulness and Wheel running on the other hand was never complete -- sleep was often interspersed throughout the active phase and Wakefulness, throughout the inactive phase. The most striking phenomena were breakdowns of this inter-relationship between sleep and activity. Such breakdowns were characterized by a damped, relatively arrhythmic sleep rhythm coexisting with a well-defined, highly rhythmic activity rhythm. No environmental conditions could have accounted for such sleep rhythm disturbances. Yet, within mice over time, some recordings showed disruptions and others did not. Based on recent lesion work with the suprachiasmatic nucleus, one explanation of the phenomena is that the activity and the sleep rhythms have separate, dissociable suprachiasmatic pacemakers. Thus damping of the sleep rhythm may result from a phase relationship between the two pacemakers such that the peak-trough pattern in the sleep rhythm was blocked by competing activity.

A program on a PDP11T34 computer operating with RSX-11M has been devised to monitor continuously and on-line all four variables (Wakefulness, NREM sleep, REM sleep, and Wheel running) so that longterm interrelationships among the rhythms can be evaluated in association with any disruption in sleep rhythms. The program makes decisions using a conditionally adaptive, template matching algorithm. For each 10 seconds, digitized electrocorticographic data are used in conjunction with yes-no information from a wheel running detector. Handling more than one animal at a time is possible, since the configuration uses 15% of CPU time per mouse.

1501 RETROGRADE TRANSPORT STUDIES OF RELATIONS BETWEEN RAPHE NUCLEI AND LOCUS COERULEUS. Peter J.Morgane, William B.Forbes and Daniel A.Pasquier (SPON: W.L.McFarland). Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

In order to elucidate the possible circuitry linking the raphe nuclei with the dorso-lateral pontine tegmentum, we carried out retrograde transport studies of horseradish peroxidase (HRP).

In the rat,HRP solution(Sigma type VI;30 to 50% in saline) was injected through an 80-100 micron diameter micropipette into the dorsal raphe nucleus(DR)or the locus coeruleus(LC).An injection volume of 0.05 microliters was used.In 20 rats the injection was placed in the DR;in 12 of these the HRP was restricted to the target nucleus.In four other rats,HRP injections were made specifically in the main part of the LC.In control animals HRP injections were made in the cerebellar cortex,the oculomotor complex or the colliculus superior.Also,HRPinjections in the DR after suction of the cerebellar cortex were made.In addition,injections of 0.1 microliter of a similar HRP solution were made in the cat LC,through a one microliter Hamilton microsyringe.As it has been already described for the cat(Pasquier & Reinoso,Brain Res.120: 540,1977)brains were removed,cut in 40 micron sections,processed in diamino-benzidine,and mounted on the same day.Sections were left overnight at 37°C,then lightly counterstained with cresyl violet,cover-slipped,and studied under bright field and dark field illumination.

In the rat,after HRP injections in the DR labeled neurons were consistently found in the parabrachialis nuclei,primarily in the dorsal one.However,labeled neurons in the LC were not found,even when HRP injections placed in the DR diffused slightly in the central gray around DR.The same result was obtained after injections performed along the rostral-caudal axis of the DR.

In the rat and the cat,on the other hand,HRP injections in the LC showed labeled neurons in the DR.These labeled neurons seemed to be localized mainly in the rostral part of the DR.Labeling of DR neurons following small or large HRP injections in the cerebellar cortex was not found.

In summary,our data demonstrated that a direct DR connection to the LC exists,which is not reciprocated.Therefore,these studies support the view that the DR relates directly to the LC and could,thereby,exert influence on PGO spike release via these connections.Failure to demonstrate a LC to DR direct connection, in this material,does not favor the view that LC activity is associated with direct inhibition of the dorsal raphe nucleus.

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1502 SLEEP STATE EFFECTS UPON RESPIRATION FOLLOWING VAGOTOMY AND CORD TRANSECTIONS IN THE CAT. A. Netick*, A. S. Foutz, and W. C. Dement. Department of Psychology, California State University, Hayward, CA 94542 and Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305.

Breathing in the cat, slow and regular during NREM (non-rapid eye movement) sleep, speeds and becomes irregular during REM sleep. To determine whether this effect of state is dependent upon peripheral mechanisms, a series of 8 cats was prepared with standard electrode arrays for determining sleep stage. In addition, a tracheostomy in each cat permitted the insertion of a cannula attached to a pneumotachograph for recording respiration, and head-bolts imbedded in acrylic skull cap were used to restrain atraumatically the cats during recording sessions. Experiments were tape recorded; extensive samples of breathing under REM and NREM sleep were later digitized and analyzed.

Four of the cats were prepared for chronic recording by exteriorizing the cervical vagoaortic-sympathetic trunk in loops of skin; the nerve was inactivated by circulating cold alcohol through copper radiators around the skin loops. The remaining 4 cats were prepared semichronically with cord transections at T1; these cats later sustained bilateral vagotomies.

Inactivation of the vagi eliminated neither the relative speeding nor irregularity of breathing during REM sleep. Instead, the effect was exaggerated since the typical slowing of the vagotomy primarily affected breathing during NREM and not during REM. Following the cord transection eliminating intercostal breathing as well as peripheral feedback from the thoracic area, the state effect persisted as it did when this lesion was combined with the bilateral vagotomy.

It may be concluded that the effect of REM sleep on the frequency and regularity of breathing cannot be ascribed to afference mediated by the vagal nerves, nor to innervation of intercostal muscles, nor to intercostal to phrenic reflexes. This finding suggests an independent central origin for the effect of state upon breathing in the cat.

(Supported by NIH grant #NS 10727 and NIH Research Scientist Award MH 05804 to WCD.)

1503 OXOTREMORINE-INDUCED TYROSINE HYDROXYLASE ACTIVATION AND PARADOXICAL SLEEP IN THE RAT. P. Ramm* and H. Taub* (SPON. M.W. Donald). Queen's Univ., Kingston, Ont. K7L 3N6. Oxotremorine, a cholinersic agonist which is believed to cause prolonged elevation of tyrosine hydroxylase (TH) activity in the region containing the locus coeruleus (LC) (Lewander et al.; Nature 258: 440, 1975), was administered (1.5 mg./ks.) to seven rats. Sleep-wake activity was monitored (22 hr./day) for 15 days subsequent to a single injection of oxotremorine. This time period included the period of TH induction previously reported. Assays of brainstem TH activity confirmed enhanced activity 72 hrs. after oxotremorine administration. Minor alterations in sleep-wake activity occured during the period of enhanced TH activity, however no quantitative changes in paradoxical sleep (PS) were observed. These data are inconsistent with theories suggesting that: (a) noradrenersic neurons of the LC are directly responsible for executive regulation of PS, and (b) PS functions to maintain synaptic availability of catecholamines. These data suggest that, if a noradrenersic arousal system is present, it does not substantively affect electrocortical arousal during PS.

1504 ACTIVITY OF HUMAN HIPPOCAMPAL FORMATION AND AMYGDALA NEURONS DURING SLEEP. Luigi Ravagnati*, Eric Halgren, Thomas L. Babb, and Paul H. Crandall*. Dept. Surg./Neurol., Sch. Med., UCLA, Los Angeles, CA 90024.

Fine wire microelectrodes were bilaterally implanted in the hippocampus (HC), hippocampal gyrus (HCG), and basolateral amygdala (AM) of 17 psychomotor epileptics for diagnostic purposes. Action potentials from a single or a few neurons (termed a "unit") were recorded during a single 4-8 hr. nocturnal session including wakefulness (AW) and natural sleep. Sleep stages were scored according to standard EOG, EMG and EEG criteria. Visual inspection of the entire sleep study, and computer analysis of representative epochs, yielded the following conclusions: 1) Of the 107 units recorded during AW and different stages of sleep, 81% showed significantly ($p<.01$) different firing rates in different periods. 2) 69% of the 74 units recorded during a complete sleep cycle showed a significant change in firing rate at both the transition from AW to slow-wave sleep (SWS), and from SWS to REM sleep. In 80% of these responsive units, the change in firing rate at the AW→SWS transition was opposite to that at SWS→REM (Fig. 1). 3) HC neurons characteristically increased their firing rate during SWS and decreased it during REM, whereas most HCG neurons, the principle input of the HC, had the opposite pattern (Fig. 2). 4) Interval histograms and autocorrelograms generally did not change between sleep stages. However, some units did show a preponderance of shorter intervals during non-REM sleep, especially in ST3 and ST4, indicating a more 'clumped' or 'bursting' pattern of firing during these periods. 5) EEG spike-wave complexes characteristic of epileptic activity, if present, were greatest during non-REM sleep, and were suppressed during REM sleep, usually to levels below AW. Most units did not appear to change their firing in relation to epileptic spikes. Changes in firing rate that may possibly have been due to changes in the rate of epileptic EEG spikes are excluded from the above analyses.

We interpret our results as suggesting that the tonic levels of HC firing are not controlled by the HCG. They may instead be due to activity in septal or monoaminergic afferents. In all structures, firing rates during REM resembled those during AW rather than SWS. (Supported by the Italian CNR, by USPHS Grant NS 02808 and by the Ralph Smith Foundation)

Fig.1:All Structures

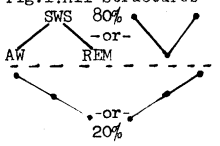

Fig.2	SWS>REM	SWS<REM	no change	total
HC	20	11	8	39
HCG	12	28	5	45
AM	8	8	7	23

1505 SLEEP IN AGED RODENTS. R. Rosenberg*, H. Zepelin* and A. Rechtschaffen* (SPON: B. E. Jones). Univ. Chicago, Chicago, IL 60637.

In humans, decreases in the amplitude of EEG delta activity and Stage 4 sleep begin in adolescence and continue throughout the life span. Increased awakenings at the end of the night and daytime naps result in a decrease in the day/night difference in amount of sleep in aged humans. EEG amplitude and diurnal rhythms of sleep in young and old F-344 rats and C57BL/6 mice were compared to determine if changes which occur with age in humans are present in rodents. Polygraph recordings of rat EEG and EMG were automatically scored for sleep stage; mouse recordings were visually scored. Resetting integrators were used to quantify unfiltered and prefiltered EEG amplitude (at 2-4, 6-8, 12-15 and 20-40 Hz for rats; at 2-4, 4-8, 8-16 and 16-32 Hz for mice). Zero-crossing wavelength detectors were used to count the number of delta (2-4 Hz) waves reaching a criterion height level (80 μv for rats, 25 and 168 μv for mice).

No significant age-related loss of EEG activity in any stage or frequency band was found in aged rats. In contrast, old mice showed a nonsignificant tendency toward lower EEG amplitude in all frequency bands and significantly fewer ($p<.05$) 168 μv criterion delta waves. Some loss in amplitude occurred in all stages in aged mice.

The diurnal distribution of NREM sleep and the diurnal distribution of delta activity within sleep (i.e., in young rodents there is more delta activity within sleep which occurs in the light part of the cycle) did not change in amplitude with age in mice. Aged rats, however, showed significant decreases ($p<.05$) in both the amplitude of the sleep rhythm and of the diurnal rhythm of delta activity within sleep as compared with young rats. Sleep bouts were also significantly shorter ($p<.05$) in old rats.

The results indicate that old mice are similar to humans in demonstrating an age-related decrease in EEG slow wave activity whereas old rats are similar in demonstrating decreased amplitude of diurnal sleep rhythms. The fact that sleep EEG amplitude in rodents may remain stable while diurnal rhythms change, and vice versa, indicates that different mechanisms govern these parameters. The loss of diurnal rhythms following lesion of the suprachiasmatic nucleus in rats suggests that the attenuation of diurnal rhythms in aged humans may be associated with some impairment of this nucleus. It has been suggested that the age-related loss of cortical neurons in humans may be responsible for the decline in delta activity amplitude. Since the neuronal population in both rats and mice is reportedly stable throughout life, the difference between the two rodent species reported here is evidence inconsistent with this hypothesis.

1506 EFFECT OF SLEEP DEPRIVATION ON LYSOSOMAL ENZYME ACTIVITY. Martin J. Skoczylas and Arabinda K. Sinha, Dept. Physiology, CMDNJ-Rutgers Med. Sch., Piscataway, N.J. 08854

In order to test the hypothesis that catabolism of nervous tissue increases during sleep deprivation, male adult Golden Syrian hamsters were hand-deprived of sleep (6, 12, & 18 hours) and the specific activities of three lysosomal enzymes, cathepsin D, β-N-acetylglucosaminidase, and acid phosphatase, were assayed in 0.32 M sucrose homogenates of cerebral cortex, pons and medulla and the rest of the brain, "midbrain". All animals were killed at about noon. By the 18th hour, the mean (12 animals in each group) specific activity of cathepsin D decreased by 25%, 23% and 21% of the control, respectively, in the cortex, "midbrain" and pons and medulla. The mean β-N-acetylglucosaminidase specific activity decreased only in cortex (22% at 18th hour), while the mean acid phosphatase specific activity decreased only in the "midbrain" (21% at 18th hour). The experiment was repeated by sleep depriving the animals by a mechanical device. The results were very similar to hand deprivation. These decreases in total specific activity following sleep deprivation may reflect an overutilization of the pools of these enzymes, whereby, their degradative rates exceed their rates of synthesis. The possibility of an increase in the concentration of inhibitors to the enzymes assayed is currently under investigation. (supported by an institutional GRS grant and ONR N00014-77-C-0332).

1507 EYE MOVEMENTS AND BLINKING IN SCHIZOPHRENIA: NEUROPHYSIOLOGIC CONSEQUENCES AND POTENTIAL FUNCTIONS OF BLINKING AND REM. J. R. Stevens, A. Livermore*, K. Wetzel* and S. Rosenblum* Depts. of Neurology, Psychiatry and Psychology, University of of Oregon Health Sciences Center, Portland, OR 97201.

Striking abnormalities in spontaneous blinking and lateral eye movement have been observed on clinical examination and recorded by telemetry from unmedicated patients with schizophrenia. These include unwinking stare, rapid rhythmic blinking, spontaneous rhythmic saccades and searching ("checking") movements. Systemic administration of dextroamphetamine (1 mg/kg) and apomorphine (0.5 mg/kg), threshold doses for behavioral response, and instillation of the GABA blocking agent bicuculline in the ventral tegmental area of the cat are associated with similar changes in eye movement. Spontaneous blinking has a relatively constant rate in all mammals which is independent of heat, humidity or the integrity of the afferent innervation of lids and cornea. Blinking during waking and REMs during sleep expose the peripheral retina to periodic brief pulses of light which provide a series of impulses, the number of which is directly proportional to the duration of waking and paradoxical sleep cycles. There is evidence that the alternating slow and paradoxical phases of sleep are associated with cyclic changes in release of hypothalamic-hypophyseal factors which regulate a number of metabolic and endocrine cycles, and that light conducted over direct pathways from retina to hypothalamus and mesencephalon contribute to regulation of these cycles. Experimental and field data will be presented which are consistent with the relationship between REM and blinking in a number of species, and which suggest that these ocular events excite oculo-endocrine pathways which regulate biologic activity in relation to the photoperiod.

1508 SEX DIFFERENCES IN THE ONTOGENY OF SLEEP APNEA DURING THE FIRST YEAR OF LIFE. Evelyn B. Thoman, Margaret P. Freese*, Patricia T. Becker*, Christine Acebo*, Victor N. Miano*, and William D. Tynan*, Department of Biobehavioral Sciences, Univ. of Conn., Storrs, CT 06268.

Respiration and behavioral sleep states were recorded for 15 male and 13 female infants over the first year of life. The first observation was made during an interfeeding period in the hospital at 2 days of age; then observations were made in the home during a 7-hour period whenever the infants were put down to nap, on weeks 2, 3, 4, and 5; and finally, observations were made in the home during the first two hours of overnight sleep at 3, 6, and 12 months of age. From the analog recordings of respiration, all apneic episodes, or respiratory pauses, of two seconds or longer were measured. Analyses of these data were made for active and quiet sleep states separately.

During active sleep, female infants had higher frequencies of apneic episodes and greater total duration of apnea during observations at all ages through 6 months of age; during quiet sleep females had greater frequency and duration of apnea through 3 months of age. The two sexes did not differ with respect to the mean length of apneic episodes in either sleep state. However, females had longer single apneic episodes in both sleep states throughout the first year.

The two sexes also differed in their predictability of apnea levels. Female infants were predictable at all ages from one observation to another throughout the first year of life. For the male infants, there were significant correlations within the first 5 weeks and between 6 months and 12 months; however, the correlations from 5 weeks through 6 months were not significant.

These results indicate a differential ontogeny of apnea in male and female infants which is most prominent during the same age period that SIDS incidence is highest. The females in this study had higher levels of apnea and more predictable apnea than the males through 6 months of age. We also know that males are more susceptible to SIDS, thus suggesting that their lower levels of apnea and/or their unpredictability may be associated with their greater risk for SIDS. Other recent evidence supports this position.

1509 AN IN VIVO MANIFESTATION OF TEMPORARY DECREASE OF INHIBITIONS IN VISUAL OR AUDITORY NEURON SYSTEMS. Clara Torda. Mt.Sinai Sch.Med & N.Y.Center for Psychoanalytic Training, N.Y.,N.Y.,10029.

It has been observed that selective inhibitions of inhibitory pathways of different sensory modalities may occur during differential cooling of thalamus. Evoked potential studies revealed that these periods of inhibition concur with increased amplitudes of peaks of the evoked potential of the same sensory modality. It is also known that the various states of consciousnes differently affect the peak amplitudes of visual and auditory evoked potentials. The amplitude of various peaks of the N_1-N_4 wave complex of the auditory evoked potentials are smallest during the fast wave sleep(the period of visual dreams). On the other hand, the amplitude of various peaks of visual evoked potentials are equal or larger than during wakefulness. It occurred, therefore, to study the nature of dream content and the nature of concurrent sensory evoked potentials. Ten adult volunteers (both sexes) participated in the experiments, several nights each. Preliminary experiments were first performed during several nights consisting of: a) recording visual and auditory evoked potentials during the various sleep phases; and b) recording of dreams during fast wave sleep. Evoked potentials were recorded by means of silver disk surface electrodes on a Grass polygraph and were fed into a compiuter of Average Transients (Mnemotron CAT 400). During other experimental nights the subjects were awakened by a taped instruct of increasing sound-intensity, stating: "please, tell out loud what goes through your mind right now", repeated until the subject awakened. The verbal reports were taped and the contents were evaluated by two independent judges. When awakened during fast wave sleep, most subjects reported dreams with visual content, and two subjects were found who reported dreams of auditory modality. Concurrent records of auditory evoked potentials revealed that during dreaming with auditory content the auditory evoked potentials resembled records obtained from states of wakefulness, during dreaming with visual content the auditory peaks had much smaller amplitudes. Also, when subjects habitually dreaming in imagery were awakened during sleep periods with auditory evoked potentials of high amplitudes, if they reported dreams, they were of auditory content. Comparison of these results with several types of control measurements led to the conclusions that: I) Individual variations occur in the incidence of interrelationships of inhibitory and excitatory processes of a sensory modality; and II) Changes of the ratio of excitatory and inhibitory processes within one sensory modality may affect the sensory mode of the dream content.

SOMATOSENSORY SYSTEMS

1510 PRIMARY AND NONPRIMARY SPINAL AFFERENTS TO THE GRACILE - Z NUCLEAR COMPLEX IN GALAGO. B. C. Albright* (SPON: D. E. Haines). Department of Anatomy, Medical School, University of North Dakota, Grand Forks, North Dakota 58201.

Individual dorsal rhizotomies were made unilaterally at representative spinal segments in seventeen bushbabies. The dorsal quadrant of the lateral funiculus was transected unilaterally at high cervical levels in two additional specimens. The medullae were sectioned in either transverse or horizontal planes and stained with either cresyl violet or Fink-Heimer methods.

The caudal pole of nucleus Z is present at levels through the obex. At this level, it is recognized as a small compact cell group which is contiguous with the ventrolateral border of the gracile nucleus. Rostrally nucleus Z shifts medially and is present along the ventromedial border of the nucleus gracilis. Rostral to the obex, the gracile nucleus decreases in size, becomes more diffuse and ends about 0.5 mm rostral to the obex. Rostral to nucleus gracilis, nucleus Z shifts dorsolaterally and terminates dorsal to the caudal portion of the spinal vestibular nucleus. Nucleus Z has two distinct longitudinal cytoarchitectural regions. At levels where both gracile and Z nuclei are present, their borders are contiguous and nucleus Z is composed of a compacted arrangement of small round and ovoid cells. Rostral to the gracile nucleus, Z is characterized by a diffuse arrangement of small cells of various multipolar shapes.

Lesions of the lateral funiculus produced dense localized preterminal debris throughout both the compact and diffuse regions of nucleus Z. Sparse contralateral debris was present in both the gracile and caudal Z nuclei. Just caudal to the obex, lateral funiculus lesions produced dense localized degeneration in the base of the gracile nucleus and sparse to moderate diffuse debris in dorsal areas. Sparse to moderate diffuse degeneration was present in the gracile nucleus at rostral pole levels. Dorsal root lesions at coccygeal, sacral and low to mid-thoracic levels resulted in moderate to sparse degeneration within the gracile nucleus rostral to the obex. Of these, sparse degeneration from coccygeal and sacral lesions was present in the medial part of the nucleus Z compact cell region. Dorsal rhizotomies at C_2, C_6, C_7 and T_1 segments produced moderate to sparse degeneration within overlapping fields in the ventrolateral part of the caudal Z nucleus. No primary afferent preterminal debris was present in the diffuse cell region of nucleus Z.

1511 EFFECTS OF EARLY WHISKER DAMAGE ON THE THALAMUS OF THE MOUSE: NEGATIVE EVIDENCE FOR "COMPETITION" IN THE SOMATOSENSORY SYSTEM. J.R. Anderson* and T.A. Woolsey. Dept. Anat. & Neurobiol., Washington U. Med. Sch., St. Louis, MO 63110.

In mice, damage to the vibrissae in the first week of life leads to alterations in the normal cytoarchitectonic patterns of barrels to which they project in SmI cortex. These patterns are precisely correlated with abnormal arrangements of thalamocortical projections to the barrels. The recent description of "barreloids" in the arcuate division of VB in the mouse, prompted us to look for cytoarchitectonic changes in the thalamus after whisker damage. In particular we were interested in the possibility that the thalamus - like the cortex - would show an altered cytoarchitecture and whether there was any evidence in VB which would suggest a "competition" for cortical space in the somatosensory system.

Barreloids are best demonstrated in VB of adult mice when the dorsum of the hemisphere is rolled 30° laterally and its anterior aspect elevated 2° with respect to the horizontal plane. Serial sections were taken through the thalami of the same hemispheres described by Woolsey & Wann '76 (vibrissa row C damaged on PND's 1-5,7,10,etc.) at 50 μm and stained with thionin. Each section containing VB was photographed. An optimal section in the series was identified and all neurons in 80 μm wide traverses across VB, perpendicular to the barreloid rows, were drawn under oil at a final magnification of 2200. The cross-sectional areas of over 3000 VB neurons were measured with the aid of a small computer.

The results are: 1) Early vibrissal damage alters the normal cytoarchitectonic arrangement of barreloids in the contralateral thalamus. 2) In appearance and time course the thalamic pattern parallels similar changes in the cortex: there is no apparent abnormality in animals lesioned on PND-7 or afterward and barreloids in rows adjacent to the areas associated with the damaged row C whiskers are larger. 3) The normal distribution of VB neuron sizes reveals two sharp peaks: the smaller neurons (15%) averaging 25 μm^2; the larger neurons (85%) averaging 85 μm^2. 4) A careful comparison of neuronal sizes across VB, especially in the altered zones associated with row C, shows no significant differences in mean neuronal sizes or overall size distributions.

We conclude that there are close parallels in the pattern and time course of thalamic and cortical cytoarchitectonic alterations in response to damage of peripheral sense organs and that competition as a mechanism to explain the altered thalamocortical projections in mice with early vibrissal damage can be ruled out. (Supported by NIH Grant NS 10244)

1512 RAPHE-SPINAL NEURONS: RESPONSES TO SYSTEMIC AND INTRACEREBRAL OPIATES. Stuart D. Anderson* and Howard L. Fields. Dept. Neurol. and Physiol., Sch. Med., UCSF, San Francisco, CA 94143.

In decerebrate or chloralose-anesthetized cats, dye-filled glass micropipettes were used to record single neurons in nucleus raphe-magnus (NRM) of the medulla (Taber et al., J. Comp. Neurol. 114:161, 1960). Bipolar stimulating electrodes were placed over the dorsolateral funiculus of the cervical spinal cord. NRM neurons were identified as projecting to spinal cord by their fixed latency antidromic spike elicited from one DLF electrode which collided with an orthodiomically conducted spike.

Fifty-nine neurons located with NRM were identified as raphe-spinal. The conduction velocities of these neurons ranged from 12^{5} to 60^{7} m/sec, the mean was $30^{5} \pm 11^{8}$ m/sec.

Raphe-spinal neurons were tested with brief trains of stimuli through electrodes implanted in the periaqueductal-periventricular gray regions. Of 18 raphe-spinal neurons tested, 14 were excited and 4 unaffected. Most raphe-spinal neurons had receptive fields similar to those reported for unselected cells in NRM (Brain Res. 123:363, 1977; Exp. Neurol. 53:304, 1976). Raphe-spinal cells usually respond to noxious or innocuous mechanical stimuli or to noxious heating of the skin or to auditory stimuli. Of 41 raphe-spinal neurons tested, 31 showed increased activity to mechanical or thermal stimuli in the noxious range.

The effect of opiate injected systemically or directly into midbrain on the activity of 13 raphe-spinal neurons was studied. Naloxone-reversible excitation was produced by systemic opiate (≤ 2.2 mg/kg morphine sulfate) in 3 neurons and by direct midbrain injection (≤ 15 μg etorphine or morphine) in 3 other neurons. Three of five neurons with naloxone-reversible opiate-induced discharge acceleration were also excited by noxious peripheral stimuli.

These results are consistent with the hypothesis that NRM is part of an endogenous pain-suppression system which mediates opiate analgesia by inhibiting spinal pain-transmission neurons (Mayer and Price, Pain 2:379, 1976).

1513 EFFECT OF IONTOPHORESES OF MORPHINE AND NALOXONE IN THE PERIAQUEDUCTAL GRAY ON THE ACTIVITY OF THE CELLS IN THE NUCLEUS RAPHE MAGNUS. Michael M. Behbehani. University of Cincinnati, College of Medicine, Dept. of Physiology, Cincinnati, Ohio 45267 USA.

The experiments reported here were designed to determine whether direct injections of morphine or naloxone in the periaqueductal gray (PAG) can alter the activity of the cells in nucleus raphe magnus (RM). Rats were anesthetized with ether and after cannulation of the femoral vein were maintained under urethane and α-chloralose anesthesia. A twin electrode consisting of 200mM naloxone hydrochloride and 50mM morphine sulfate was inserted in the PAG and another twin electrode consisting of a recording electrode and a 0.5M sodium-L-glutamate electrode was inserted in the RM. After isolation of a stable single unit in the RM, its response to either morphine or naloxone iontophoresed in the PAG was examined. The activity of those cells in RM which had very slow or no spontaneous activity was increased by iontophoresis of glutamate in the area surrounding the recording electrode. Morphine increased the spontaneous or glutamate-induced activity in 40% of the cells, an effect which was reversible by naloxone. Injection of naloxone before morphine decreased the firing rate of these cells and this effect was reversible by morphine. In approximately 20% of the cells, the opposite phenomenon was true. Both morphine and naloxone effects were detectable within one minute after their injection in the PAG. The morphine effect outlasted the injection period whereas the naloxone effect stopped within a few seconds after the naloxone injection was stopped. Some cells responded similarly to both morphine and naloxone and the remainder of the cells were not affected by either drug.

1514 ELECTRICAL AND THERMAL SENSITIVITY OF PERIPHERAL NERVE FIBERS. Spencer L. BeMent. Bioelectrical Sciences Laboratory, University of Michigan, Ann Arbor, MI 48109

Glass capillary microelectrodes were used to record from single primary afferent fibers in the superficial radial nerve of anesthetized cats maintained at 36-38° core temperature. The nerve was stimulated with bipolar hook electrodes 40-70 mm from the recording site and with natural stimulation of the forepaw receptors. The temperature of the mineral oil or saline-filled well connecting the stimulating and recording sites was carefully controlled with heating wires embedded in the paw holder. The conduction velocity was obtained from the latency of each single fiber response to supramaximal stimulation. The electrical stimulus was a short duration (20-50 μsec) constant current pulse. The pulse amplitude was obtained with a current probe. The data were taken at well temperatures from 28 to 36°C and corrected to specific temperatures with a Q_{10} value of 1.8 (Paintal, J. Physiol. 180:20, 1965).

The stimulating current for threshold stimulation of 45 fibers in one nerve was essentially constant within statistical variation for fibers with conduction velocities between 60 and 90 m/sec whereas it increased monotonically with decreasing conduction velocity for fibers with conduction velocities between 25 and 35 m/sec. No fibers were recorded with conduction velocities in the 35-60 m/sec range in this nerve. The threshold stimulation results for fibers with conduction velocities greater than 60 m/sec are at variance with the popular view that the larger the fiber diameter the less the current required to stimulate.

The conduction velocity histogram from 178 fibers in six nerves corrected to 37°C exhibited peaks at 25-30 and 60-65 m/sec; no fibers were found that conducted between 40 and 50 m/sec. However at 28°C the histogram peaks were at 15-20 and 45-50 m/sec; the faster fiber peak then appeared within the conduction velocity region where no fibers were found at 37°C. Therefore, the temperature of the nerve segment between the stimulating and the recording sites must be taken into account to avoid thermal smearing of conduction velocity determinations, even when the core temperature of the animal is maintained relatively constant. This fact is particulary important in studies where receptor types are related to conduction velocity (e.g. Bromberg and Whitehorn, Brain Res. 78:157, 1974; Burgess et al., J. Neurophysiol. 38:833, 1968)

The single fiber action potential duration was found to increase with decreasing conduction velocity in agreement with results (Paintal, J. Physiol. 184:791, 1966) for individual fibers recorded from filaments of cat saphenous nerve.

(Supported by USPHS Grant NS 08470)

1515 MAGNITUDE ESTIMATES OF SPATIAL SUMMATION FOR CONDUCTED COOL STIMULI. Sherry L. Berg* and Dan R. Kenshalo. Dept. Psychology, Florida State University, Tallahassee, Florida 32306.

The psychophysical method of magnitude estimation was used to measure spatial summation of cool stimuli in five human observers. Contact thermal stimuli were presented to the right dorsal forearm after the site had been preadapted to 32.6°C. Twelve rates of temperature change ranging from 0.1° to 2°C/sec were administered for a 3 sec duration producing twelve corresponding logrithmically spaced cooling shifts from 0.3° to 6°C. Five stimulating surfaces (1,2,4,7.5 and 18cm^2) were introduced using all twelve intensity levels so that spatial summation for thermal cooling could be assessed.

The uniform shape and identical slopes of the power functions obtained for each of the five stimulating areas for the given intensity range suggests a unitary sensorineural mechanism underlying magnitude estimates of cooling. Additionally, the shallow slopes ($\beta = 1$) and the relatively restricted range of displacement for the five identical areal functions demonstrates that, in general, the absolute spatial summation at and above threshold for the cool sensation is less than that observed for the warming sense. (Supported by USPHS Grant NB-02992 and Training Grant MH 11218)

1516 PROJECTIONS FROM THE FIRST AND SECOND SOMATIC SENSORY CORTEX, THE MOTOR CORTEX AND THE CEREBELLUM TO THE POSTERIOR GROUP OF NUCLEI IN THE THALAMUS OF THE CAT. K. J. Berkley and D. C. Mash*. Dept. Psychol., Fla. St. Univ., Tallahassee, FL 32306.

The distribution within the posterior group of terminals from these various afferent sources was studied using a differential labeling strategy in which both autoradiographic and degeneration tracing methods were employed to label two different afferent pathways simultaneously in the same cat. This strategy allows one to make direct comparisons between the terminal distributions of the two afferent pathways under investigation.

In agreement with the results of other investigators, it was found that the first and second somatic sensory cortices projected to those portions of the medial division of the posterior group (POm) that also receive input from the gracile, cuneate, trigeminal and lateral cervical nuclei as well as from the spinothalamic tract. Although these projections appear to overlap completely with each other, the densest projections from the second somatic sensory cortex tend to be located more laterally than the densest projections from the first somatic sensory cortex.

In addition to the somatic sensory cortical projections and the projections from the other sources named above, POm also appears to receive inputs from the cerebellum and the motor cortex. These inputs are modest, tend to occur in not-necessarily-overlapping patches, are more likely to be found at the rostral and caudal poles of POm, and are consistently observable with both autoradiographic and degeneration labeling methods.

This highly convergent pattern of connectivity within POm is similar to the pattern that has been observed in the rostrally adjacent thin region that lies between the n. ventralis posterolateralis and the n. ventralis lateralis. This similarity suggests that these two regions may form one continuous zone that receives input from a wide variety of afferent sources with different functional characteristics and covers the caudal, dorsal, medial and rostral borders of the VPL and VPM somatic sensory thalamic nuclei.

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1517 THERMAL STIMULATION OF THE CORNEA IN HUMANS. R.W. Beuerman and D.L. Tanelian.* Div. of Ophthalmology, Stanford Univ., Stanford, CA 94305

There is at present disagreement as to whether or not touch or temperature sensations can be evoked from stimulation of the human cornea in the absence of irritation or pain. In the present experiments, a device has been developed which allows precise control of the stimulation parameters. The subject was seated with the head supported while looking downward so that the eye was immersed in the stimulation chamber containing slowly circulating sterile saline at 33°C. Stimulation was by a saline jet (250 μm diameter) emerging from a nozzle at the bottom of the chamber 2-3 mm from the eye. The jet was switched rapidly from the adapting temperature (33°C) to the stimulus temperature (28° C-45°C) for a 2 sec. period. The rise time of the stimulus pulse was 375 msec, and the reproducibility of a stimulus temperature was ±0.05°C. The jet pressure was about 5 cm H_2O, which was below mechanical threshold for all tissues examined in this study.

When temperature pulses were applied to the upper eyelid in four trained subjects, only verbal responses of temperature sensation were obtained; there were no reports of irritation or pain. Using the same subjects, the experiment was repeated with the eye open so that the jet would impinge on the center of the cornea. All subjects reported sensations of irritation or pain as well as those of temperature. Irritation was initially reported at 35°C, while pain was not felt until 42°C. The sensations of irritation or pain were brief; a clinical examination found that the cornea had not been injured. Since it was found by visualizing the jet fluid with fluorescein that the lid margins were also contacted by the stimulus, additional experiments were designed to determine which sensations were evoked by thermal stimulation of the cornea. In the first experiment a specially designed hard contact lens covered the entire cornea; in the second the cornea was anesthetized while the lids retained normal sensation; and in the third experiment the cornea was mechanically isolated from the lid margins by a soft rubber cup which contacted the eye posterior to the cornea. In the first two experiments, the threshold for temperature sensations were similar to those previously determined while irritation was not reported. In the last experiment irritation was sensed at 36°C and pain at 42°C; temperature sensation was absent. It is concluded that although thermal stimuli are adequate stimuli for corneal receptors, only sensations of irritation or pain are evoked and that this sensory pathway may only have a nociceptive function.

(Supported by NIH Grants EY 00431 and EY 00051)

1518 STRESS-PRODUCED ANALGESIA AND MORPHINE-PRODUCED ANALGESIA: LACK OF CROSS-TOLERANCE. Richard J. Bodnar, Dennis D. Kelly, Solomon S. Steiner*, and Murray Glusman. New York State Psychiatric Institute and City College of New York, N.Y.

Rats subjected to acute stressors such as a forced cold-water swim or repeated footshock demonstrate increased nociceptive thresholds. Repeated exposure to the same stressful situation results in adaptation in much the same way that repeated administration of a constant dose of morphine results in tolerance. The present study investigated the possibility of cross-tolerance between stress-produced analgesia and morphine-produced analgesia. Ten separate groups of six rats each were tested using the flinch-jump procedure 30 min after the following experimental manipulations. In the first group, each rat was subjected to a single cold-water swim at 2°C for 3.5 min; subsequent flinch-jump thresholds indicated that the animals developed profound analgesia. In the second, third, and fourth groups, the rats were given a single injection of morphine at one of three dose levels: 15, 10 or 5 mg/kg. All three dose levels produced analgesia; the analgesia caused by the acute cold-water swim was most comparable to that produced by 10 mg/kg of morphine. The fifth and sixth groups were subjected to either a daily cold-water swim or a daily injection of morphine (10 mg/kg) for 14 consecutive days. The return of flinch-jump thresholds to base-line levels at the end of the 14 day period indicated that adaptation or tolerance had developed to both procedures. In the seventh group, the animals were subjected to cold-water swims for 13 days and then injected with morphine (10 mg/kg) on the 14th day; subsequent flinch-jump thresholds demonstrated profound analgesia rather than adaptation or tolerance. Similar results were found in an 8th group tested for reverse cross-tolerance in which chronic morphine administration was followed by an acute cold-water swim. The remaining warm-water swim and placebo control groups confirmed these results. (Supported by NIMH Grant #13579, and by New York State Health Research Council Grant #365.)

1519 SIMILAR THALAMIC PROJECTIONS OF THE ROSTRAL AND CAUDAL PARTS OF THE DORSAL COLUMN NUCLEI IN THE RAT. A.C. Bonduki* (SPON: J. I. Johnson, Jr.). Departments of Psychology and Biophysics, and Neurosciences Program, Michigan State University, East Lansing, MI 48824.

In order to determine if rostral and caudal portions of the dorsal column nuclei have different projections to the thalamus, lesions were made in the gracile and cuneate nuclei of the rat. The lesions were restricted to regions rostral to, or caudal to, the obex. Following survivals of two to seven days, the animals were perfused and the brains were processed according to the Fink-Heimer method for anterograde degeneration. Degenerating terminals were present in the ventrobasal complex pars externa, the anterior pretectal nucleus, the medial division of the medial geniculate nucleus, the ventral portion of the zona incerta, and nucleus angularis (M. Rose: Mém. Acad. Pol. Sci. Lett., Cracovie, sér. B, 1935, 1-108; W. R. Mehler: Ann. N.Y. Acad. Sci., 1969, 167, 424-468) which occupies a small region between the ventrobasal complex and the central lateral nucleus and appears as a dorsolateral extension of the latter. Terminal degeneration was not observed in any of these nuclei following exposure of the medulla without a lesion (sham operations), or following eye removals, although in the latter case degeneration was present in the nuclei of the visual pathway. This pattern of terminal degeneration in the thalamus was identical for rostral and caudal lesions. The results differ from those of Lund and Webster in the rat (J. Comp. Neurol., 1967, 130, 301-312), but are in agreement with the findings of Boivie in the cat (Brain Res., 1971, 28, 459-490) and RoBards and Watkins in the opossum (Neurosci. Abstr., 1975, 1, 215). In addition, terminal degeneration in the ventral tier of the dorsal thalamus resulting from spinal hemisections and anterolateral cordotomies (at C_3) was located rostral to that resulting from dorsal column nuclei lesions, probably in the ventrolateral nucleus.

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1520 CAT HINDLIMB DERMATOMES WITH SINGLE-UNIT RECORDING. Paul B. Brown and Herman R. Koerber*, Physiology & Biophysics Dept., West Virginia University Medical Center, Morgantown, WV 26506.

Dermatomes of dorsal roots L_4 - S_2 were determined by recording from dorsal root ganglion cells with myelinated afferents responding to light touch, using stainless steel microelectrodes.

RESULTS: The dermatomes determined with this method were smaller than those reported by previous authors. They displayed less overlap of adjacent dermatomes. Dermatomal trajectories were similar to those previously reported. Dermatomes were more similar to the corresponding dorsal horn dermatomes than are those of previous reports.

Individual receptive field sizes vary inversely with distance from toes; receptive field shapes (length/width ratios) show little variation as a function of position, averaging 1.76, substantially less than dorsal horn cells. Computed innervation density is greatest at the toes.

CONCLUSIONS: (1) previously reported dermatomes, determined using whole-root recordings, are probably inaccurate; (2) dorsal horn dermatomes differ significantly from dorsal root dermatomes, predominantly in being larger (convergence); (3) skin stretch during development probably affects dorsal horn cell receptive field shape more than primary afferent receptive field shape; and (4) previous assumptions of maximum innervation density on foot (especially toes) are tentatively confirmed by these data.

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1521 ROSTRALLY AND CAUDALLY PROJECTING NEURONS IN THE DORSAL COLUMN NUCLEI OF THE DOMESTIC CAT. M. B. Bromberg and A. L. Towe. Dept. Physiology & Biophysics, U.W. Sch. Med., Seattle, WA 98195

In the course of a population study of the cuneate nucleus and adjacent tissue in chloralose-anesthetized domestic cats, a few neurons with unusual properties were encountered. The hunting stimulus was a brief shock to the dorsal funiculus at C3, ipsilateral to the site of recording. Stimulating electrodes were also placed in the central footpad of each paw, in caudal nucleus VP-L of the thalamus, and across the lateral tip of the cruciate sulcus of each cerebral hemisphere. A few neurons were apparently driven antidromically from the dorsal funiculus electrode, and some of these were also driven antidromically from the thalamus. Among some 1700 neurons examined (most, retrospectively), only one percent could be convincingly identified as projecting caudally, with about half of these also projecting rostrally to the thalamus. Collision tests were performed on only a few of these, toward the end of the study. C3 antidromic latency ranged from 0.6 to 2.3 msec (the latter neuron satisfying the collision test); VP-L antidromic latency ranged from 1.0 to 1.6 msec. Spike splitting usually appeared at iterative stimulus rates above 200-500 per second. About half of the neurons that projected both caudally and rostrally could not be influenced by cerebral stimulation, whereas 80% of those that projected only caudally could be excited from both cerebral stimulus sites. The remaining neurons were either excited from both cortical sites, were inhibited from both, or were excited from the contralateral and inhibited from the ipsilateral cortical sites. Three-fourths of the "caudal-rostral projection" neurons were hair-sensitive, with small excitatory receptive fields typical of cuneate and gracile neurons; the "caudal projection" neurons showed no such selectivity. Fully three-fourths of these neurons were isolated along the lateral margin of the cuneate nucleus (about 2.5 mm lateral to the midline); they were isolated 0.5 to 2.5 mm below the dorsal surface. They frequently could be recorded over a long distance (0.1-0.2 mm), and had large, low-frequency spikes. C3 antidromic threshold was usually much greater than C3 orthodromic threshold, suggesting that the axons were deep in the dorsal funiculus or in the dorsolateral funiculus.

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1522 RESPONSES OF PRIMARY AFFERENTS AND DORSAL HORN NEURONS TO VAGINAL PROBING IN CATS. Mary C. Bushnell*, Michael J. Iadarola and Donald D. Price (SPON: D. J. Mayer). The American Univ. and Georgetown Univ., Wash., DC 20016 and NIDR, NIH, Bethesda, MD.

We have studied mechanisms of neural afterresponses evoked in spinal cord neurons by natural stimulation of external genitalia of female cats. Both primary afferents and dorsal horn neurons were studied in anesthetized or unanesthetized spinalized cats, respectively. Each cell was tested for afterresponses using electrical and natural mechanical stimulation. The latter included Von Frey hairs and a calibrated vaginal probe.

Several types of primary afferents responded to vaginal stimulation. One type, similar to field receptors of glabrous skin, responded to Von Frey hairs of less than 1 gm applied to the vaginal mucosa at the clitoral region. These cells showed no activity during maintained pressure and little or no response during stimulator retraction. Another type of neuron discharged only during deep vaginal-cervical probing (insertion greater than 2.5 cm) and continued to respond for the duration of the stimulus. Primary afferents with receptive fields adjacent to the vaginal mucosa often discharged when the vagina was probed. None of the primary afferents that responded to vaginal probing exhibited afterresponses to any stimulus.

S_2 dorsal horn neurons responding to vaginal probing included: (1) tactile neurons that had input from low threshold mechanoreceptive afferents, (2) wide dynamic range neurons (WDR) that had input from low threshold afferents and nociceptive afferents, and (3) nociceptive specific neurons (NS) that had nearly exclusive input from nociceptive afferents. Many neurons of all classes projected to the level of the spinocervical nucleus. None of the tactile neurons responded with afterresponses to any mechanical or electrical stimulation of the vaginal region, and the NS neurons responded with afterresponses only to distinctly noxious stimuli. WDR neurons responded with increasingly greater frequencies as the force applied to the vaginal probe was increased from 30 to 110 gm/12.5 mm^2, and 75% of these neurons responded with distinct afterresponses following the termination of vaginal probing. It appears that these tactile-evoked afterresponses of WDR neurons cannot be attributed to afterresponses of primary afferents or to descending control but are produced by mechanisms within the spinal cord. These data lend support to the conclusion of Price et al. (Neurosci. Absts., 1977) that input over WDR neurons may mediate non-noxious aftersensations.

1523 REACTION TIME TO FIRST PAIN IN HUMAN SUBJECTS. James N. Campbell, Robert H. LaMotte. Dept. Physiology, Sch. Med., The Johns Hopkins Univ., Baltimore, Md. 21205.

The monkey hand is innervated by units sensitive to gentle warming that conduct in the C fiber range. Higher temperature stimuli activate both C fiber and A-delta fibers, the latter presumably subserving the sensation of "first" pain. It was predicted on this basis that the reaction time to heat stimuli of increasing intensity would decline sharply as the stimulus temperature approached the threshold for first pain. To test this hypothesis we measured the reaction time (RT) to radiant heat stimuli applied either to the thenar eminence of the hand or the volar forearm in three human subjects. The subject was instructed to release a key as soon as the first sensation occured whether it was warmth or pain. A CO_2 infrared laser under control via a radiometer provided a step increase in skin temperature to a constant level ($\pm$ 0.1°C) over a 7.5mm diameter spot. Stimuli were of 2s duration, and were followed by passive cooling to a base temperature maintained at 38°C. Runs consisted of 40 presentations of the same intensity that ranged in value from 39 to 51°C. Eight loci, on either the thenar eminence or distal volar forearm, were stimulated in rotating sequence, and at random intervals, with a mean rise time to the desired temperature between 200 and 250msec for each stimulus value. The mean inter-trial interval was 25s, and the mean interval for stimuli delivered to the same locus was 200s. When stimuli were delivered to the thenar eminence, the first sensation was typically that of warmth, and the median RT declined exponentially with increasing stimulus temperature to an asymptote of 520-1000msec for the three subjects. For stimuli delivered to the forearm, RT declined in a similar manner in all 3 subjects to an asymptote of 750msec for temperatures of 42 to 44°C. For these stimuli the first sensation perceived was warmth. With further increases in stimulus temperature the median RT declined exponentially to a new asymptote of 400msec, and the first sensation perceived was that of pain. RT's as short as 400msec require that primary afferents conducting faster than 4m/s be activated. It is therefore likely that A-delta fibers are active in the region associated with the threshold for first pain. The response latency measure for stimuli delivered to the volar forearm may provide a measure of the first pain threshold in non-human primates, and may in addition provide a measure of the first pain threshold independent of subjective judgements in human subjects.

1524 SINGLE REPRESENTATION OF THE HAND IN SOMATIC SENSORY CORTEX (SI) OF PROSIMIAN, GALAGO CRASSICAUDATUS. Mary Carlson* & Carol Welt (SPON: Ruth Bleier). New England Reg Primate Res Ctr, Southboro, MA 01772 & Central Wisconsin Center, Madison, WI 53704.

Following the finding of multiple projections of the hand to the primary somatic sensory cortex (SI) of the rhesus monkey, we began studies of the pattern of input from the hand to the cortex of a prosimian, Galago crassicaudatus. Although an earlier study of SI in a prosimian (Nycticebus coucang), had described but one hand projection responding to low threshold (LT) cutaneous stimulation, we felt that examination of another prosimian was warranted in view of the new findings on the differentiation in simian cortex.

Using microelectrodes, we mapped SI in galago in extremely fine detail (over 100 punctures in 4 sq. mm) and found only one area responding to LT cutaneous stimulation of the hand. However, within this single area we found that the glabrous and hairy surfaces of the hand project separately to the rostral and caudal divisions of the area in two distinct somatotopically organized patterns. The serial pattern of representation of the two surfaces of the hand is reminiscent of the pattern of representation of the entire hand in the rhesus, but is in contrast to the mirror image rerepresentation of the hand and foot in Aotus trivirgatus, a new world simian. The topographic organization of neurons responding to manipulation of joints was studied in chronically prepared unanesthetized animals. Joint afferents projected to neurons that were intermingled with those responding to LT cutaneous stimulation in both the rostral and caudal divisions of the hand area. The segregation of glabrous and hairy input seen in anesthetized recording sessions was also evident in the waking animal. Galago lacks a central sulcus and the two sagittal sulci that intersect SI do not correspond to the rostral and caudal boundaries of the area. Instead, in horizontal flattened sections they form a continuous cell sparse line, or incipient sulcus, dividing the hand and face areas. The results of these studies confirm the existence of only one hand area in prosimians, and further suggest that the multiple projections of the hand in simians may have evolved by selective migration of submodality types (i.e. cutaneous, joint, etc.) with the maintenance of somatotopic organization.

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1525 RESPONSES OF CATS TO THERMAL PULSES. K. L. Casey, G. P. Frommer, T. J. Morrow* and R. G. Voss*. Depts. Physiol. and Neurol., Univ. Mich., Ann Arbor, MI 48109.

Pain research would benefit by the use of humane, quantitative tests of unlearned responses of animals to controlled natural stimuli. Accordingly, we have developed a semi-automated method for quantifying three behavioral responses of cats to thermal pulses delivered to each hindlimb. The method assumes that noxious stimuli should elicit vocalization, movement and interruption of an ongoing behavior at significantly higher probabilities than innocuous stimuli.

Restrained cats are trained to approach an illuminated food cup into which liquified liver is delivered when the cat's head breaks a photocell contact. A programmable integrated circuit controls the feeding schedule, timing, location and intensity of thermal stimuli, and activates 3 response latency counters. Stimuli are delivered by water-cooled contact thermodes; resting temperature is 38°C. In these experiments, pulse duration and rate of onset is 5 sec and 37.5°C/sec, respectively; fall time is about twice rise time. Pulse amplitudes are 43, 50, and 53°C. For 22 of the 43 15-sec food-available periods, a stimulus is initiated by food approach (contingent); 10 stimuli are non-contingent. Interstimulus interval ranges from 15 to 72 sec, and the stimulus sequence is based on a random order of intensity and laterality. Sensors for each response (movement, vocalization, and interruption of food approach) stop digital counters and register response latency. Any response can be selected to abort (escape) a stimulus.

Response probabilities of 4 normal cats to 43°C stimuli are not significantly different (χ^2 test) from those to blank trials in any response category. Three cats each gave significant responses in one of 3 different categories when pulses were 50°C. All cats, however, show highly significant food approach interrupt probabilities to 53°C pulses compared with blank trials; 3 cats also responded with movement and 2 with vocalization. These responses were maintained above control values for 3 months. Movements and vocalizations were significantly more frequent when stimuli were non-contingent. Mean interrupt latencies range from 2.72±1.17 sec to 2.34±1.42 sec and are not significantly different for movement or vocalization. A significant number of interrupt and movement responses occur within 1.0-1.5 sec, shortly after or even before final stimulus temperature is attained.

The results are consistent with thermal nociceptive thresholds determined by other methods (Rice and Kenshalo, J. Appl. Physiol. 17:1009, 1962), but suggest that a thermal rate sensitive or rate dependent process, rather than thermal displacement, initiates the behaviors monitored in this study. (Supported by NIH Grants NS12015, NS12581, and MH55989.)

1526 SOMATOSENSORY EVOKED POTENTIALS RECORDED DIRECTLY FROM HUMAN SMI CORTICAL AREA AND THALAMUS. Gastone G. Celesia. Dept. Neuro, St. Louis Univ. and Veterans Administration Hospitals, St. Louis, Missouri 63104, USA.

Recordings from the thalamus were carried out in 5 patients undergoing stereotaxic thalamotomy for treatment of Parkinson's tremor. Recordings from the exposed cortex were carried out in 4 patients undergoing temporal lobectomy for partial complex seizures.

Somatosensory evoked potentials (SEP) to median nerve stimulation recorded from VPL consisted of monophasic or diphasic potentials with mean onset latency of 13.8 msec. The major deflection was usually positive, with mean peak latency of 18.09 msec. Thalamic SEP to popliteal nerve stimulation had onset latency of 17.05 msec and peak latency of 23.3 msec.

More complex SEP to median nerve stimulation were obtained from the cortex. They consisted of two major positive waves P_1 and P_2. The mean onset latency of P_1 was 20.8 msec, the peak latency 28.4 msec. The mean peak latency of N_1 was 37.7 and of P_2 57.04 msec. SEP were recorded over both the precentral and postcentral gyri suggesting that somatosensory information converges to the motor cortex probably to be used for the integration of critical motor activity.

1527 CENTRIFUGAL CONTROL OF NOCICEPTION: AUTOANALGESIC MECHANISMS. W. T. Chance, A. C. White*, G. M. Krynock*, and J. A. Rosecrans. Dept. Pharmacol., Med. Col. Va., Richmond, Va. 23298.

Our previous reports (Proc. Soc. Neurosci., 2, 919, 1976; Fed. Proc., 36, 395, 1977) have indicated that lesion-induced hyperemotionality and conditioned fear elicit antinociception as measured by the rat tail-flick procedure. We now report the results of several experiments suggesting that these decreases in pain sensitivity are due to influences descending from higher levels in the CNS. In each of the following experiments antinociception was elicited by classically conditioning fear to the environmental stimuli associated with the tail-flick procedure. Acquisition of autoanalgesia was studied by shocking (15 sec/day; 0.8 ma) 30 albino rats 10 sec after the determination of their tail-flick latencies, while control rats (n = 20) were handled similarly but were never shocked. Although their initial responses did not differ, significant antinociception was observed in the conditioned group (4.5 vs 3.1 sec) on the 2nd test day. Furthermore, their response latencies continued to increase across the first 7 days (5.6 vs 3.4 sec; day 7). Pretreatment (30 min) with diazepam (2.5 mg/kg; i.p.) or naltrexone (1 mg/kg; i.p.) on day 8 did not reduce the antinociception (n = 10/gp). After 3 more conditioning days, the spinal cords of conditioned (n = 8) and control (n = 3) rats were severed at the T_3 level by cautery. Sham surgeries were performed on an additional 9 conditioned and 4 control rats. Assessment of antinociception on the following day revealed a significant (33%) decrease in latencies of the transected-conditioned group, while the transected control group showed a net increase in tail-flick response times.

The relationship of autoanalgesia to endogenous opiate peptides was investigated by assaying (centrifugation-liquid scintillation) binding of labeled opiates to whole brain homogenate of conditioned and control rats. In these studies binding of ^{3}H-NLeu-enkephalin and ^{3}H-etorphine was significantly reduced in conditioned rats. Furthermore, the correlation between binding and tail-flick latencies in the control rats was significant in the negative direction ($r = -0.87$; $n = 9$). Degradation of endogenous peptides by preincubation at 37° C effectively abolished the difference between groups, suggesting receptor occupation by an endogenous ligand responsible for initial differences in binding. Additional data suggesting the central nature of autoanalgesic mechanisms are preliminary experiments showing that lesions of the raphe magnus decrease antinociception in fear-conditioned rats. Since this lesion has also been reported to reduce both morphine- and methadone-induced analgesia (Fed. Proc., 36, 1024, 1977), similar centrifugal mechanisms may subserve these analgesic effects. (DA 00296-03).

1528 CELLS LOCATED IN SOMATOSENSORY CORTEX OF THE CAT SPECIFICALLY *SENSITIVE TO SKIN TEMPERATURE*. Allen B. Chatt and Dan R. Kenshalo. Dept.of Psychology, Florida State University, Tallahassee, Florida 32306.

113 cells were isolated in primary sensorimotor cortex of 20 paralyzed, unanesthetized chronic cats with 1μ, isonel coated tungsten microelectrodes. The responses of approximately 28% of the 46 cells isolated in the facial projection area were altered by alterations in skin temperature applied to their receptive fields (RF). Approximately 90% (9 of 11) of these mimicked cutaneous cold receptors in that they responded with an increase in activity to cooling stimuli. The higher level of activity was maintained until the stimulus was removed at which time the units demonstrated a decrease in activity which was on occasion followed by a rise in activity to near pre-warming levels. This level of activity then slowly decreased to pre-cooling levels. None of these units responded readily to mechanical stimuli. All RF's were large and irregularly shaped; most were contralateral to the site of stimulation, but two cells had bilateral RF's which appeared to be symmetrical and continuous across the midline. Each of these cortical temperature cells was somatotopically arranged with penetrations yielding temperature units lying near penetrations where HF and pressure sensitive units were found with similar spatial skin arrangements. Some evidence of a vertical organization of these cells was found in that 67% of the specifically sensitive cells and 46% of all thermally activated cells encountered were found in multi-cell perpendicular penetrations of cortex. Spatial convergence was a notable feature of these cells in that a large area stimulus was needed on most cells to convincingly activate the cell. Adaptation to a repeated cold stimulus was observed on a few cells. All cells were spontaneously active at skin temperatures normal for an animal under these experimental conditions. On a few cells, prior warming of the suspected RF was needed before the cell would respond to a cold stimulus. Steady state (SS) activity varied monotonically over adapting temperatures (AT) with a negative thermometer function and hysteresis was observed in the SS activity of all cells over AT. Bursting was evident at both low and high AT, but the types of bursting varied. Bursting at low AT was characterized by short interburst intervals and at high AT by long interburst intervals. The remaining cells in facial projection area responding to temperature were not submodality specific but responded to severe heating of the RF and high threshold mechanical stimuli. No units were found that mimicked cutaneous warm receptors. No units were found in forelimb projection area that were specifically sensitive to temperature although two units were found with ventral forepaw RF's that responded to severe heating and pinch. (NIH Grant NS 02992)

1529 SPATIAL SUMMATION RESPONSES OF THE CENTRAL NERVOUS SYSTEM TO UNMYELINATED AFFERENT ACTIVATION. Jin Mo Chung and Robert D. Wurster. Dept. of Physiology, Stritch School of Medicine, Loyola University of Chicago, Maywood, IL 60153.

Although temporal summation from unmyelinated afferent fibers is known to be frequently required to activate central nervous system responses, characteristics of spatial summation have not been clearly described. The possibility of spatial summation of the central nervous system to unmyelinated afferent fibers was tested using the somatosympathetic C reflex.

Adult cats were anesthetized and vagotomized, and the carotid sinuses were bilaterally denervated. Sympathetic nerve activity was recorded from the cervical sympathetic trunk while stimulating afferent nerves in the hindlimb. At a slow repetition rate, single shock stimulation of both A and C afferent fibers of a hindlimb nerve elicited a sympathetic A reflex. When trains of three pulses were given, an additional longer delayed C reflex component appeared. This sympathetic C reflex disappeared if the stimulus strength was lowered below the threshold of the afferent C fibers. These results confirm the notion that temporal summation is required to elicit the somatosympathetic C reflex. Instead of a train of pulses to one nerve, if single pulses were delivered in succession to three different ipsilateral hindlimb nerves, the somatosympathetic C reflex was also elicited. This result suggests that the somatosympathetic C reflex can be elicited by spatial summation of unmyelinated afferent fibers. Furthermore, the somatosympathetic C reflex could be also elicited by combined stimulation of ipsilateral and contralateral hindlimb nerves, suggesting bilateral spatial summation.

The somatosympathetic C reflex is known to demonstrate a recruitment phenomenon. That is, the size of the reflex progressively increases with repeated stimuli to unmyelinated afferent fibers. The recruitment phenomenon was observed during both temporal and spatial summation.

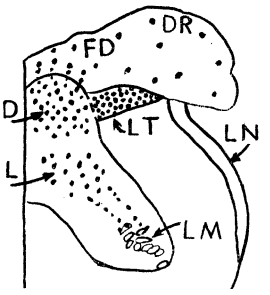
It is concluded that the central nervous system can be activated by spatial summation of unmyelinated afferent fibers as shown in the somatosympathetic C reflex. This spatial summation can be observed with bilateral afferent inputs and displays a recruitment phenomenon. (Supported by NIH Grant HL 08682.)

1530 DORSAL ROOT PROJECTIONS TO LISSAUER'S TRACT AND THE VENTRAL HORN OF THE FROG SPINAL CORD. W.L.R. Cruce, E.A. Neale, L.M. Bowers*, J.H. Neale, J.L. Barker, and B. Alexander*. Neurobiology Program N.E. Ohio Univ. Coll. Med., Rootstown, OH 44272; NIH, Bethesda, MD 20014; Anat. Dept., Howard U. Coll. Med., Washington, DC 20059.

Bullfrog (Rana catesbiana) spinal cord was removed with intact roots and maintained in a perfusing chamber at 18°C for 36 to 48 hr. Cords were viable (DR-VR reflex present) for at least 24 hr. Dorsal root ganglion (DRG) 10 was isolated through a silicone grease barrier and incubated for up to 24 hr in 500 uCi of ^{3}H-leucine in 0.5 ml Ringers. The cord was perfused with oxygenated frog Ringers containing 1mM unlabeled leucine and 20 ug/ml cycloheximide. Cords were fixed and processed for autoradiography. After 6-25wk exposure, autoradiograms were developed and stained.

Silver grains over dorsal root (DR) and dorsal funiculus (FD) fibers were sparse compared to those over spinal gray. The most dense concentration of grains was found over a region of neuropil which lay lateral to the dorsal horn. This region, which has been called Lissauer's Tract (LT) can be seen to contain fine fibers in Bodian-stained tissue and sparse myelinated profiles in osmium-stained material. In the EM, LT contains a few small diameter (0.5-2.5u) dendrites and myelinated axons as well as many fine (0.1-0.5u) profiles of axons and dendrites. Profiles of synaptic boutons are also present in about the same density as found in the dorsal horn (2-15 per 150u^2).

The second most dense region of silver grains lay over the dorsal field (D) of Ebbesson (comparable to laminae I-IV of mammals). The third greatest density lay over the lateral field (L) of Ebbesson (lam. V-VI). A clear space separated the grains over D from those over L. From the ventral edge of L, grains were distributed sparsely, though above background, all the way to the dorsal edge of the lateral motoneuron (LM) group. Frequently these silver grains were arranged in rows, suggestive of fibers. Often grains were seen gathered around the cell borders of the most dorsally situated motoneurons.



We conclude that dorsal root fibers terminate heavily in LT in the frog. Our results confirm Székely's recent demonstration (using $CoCl_2$ tracing) that dorsal root fibers can reach the somas of ventral horn motoneurons.

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1531 POSITION SENSE: THE EFFECTS OF MUSCLE CONTRACTION. A. D'Almeida* & W.Z. Rymer. Dept. Neurosurg., Upstate Med. Ctr., Syr., N.Y. 13210

Muscle stretch receptors appear to influence the perception of joint position (1)(2), although the nature and importance of their contribution remain uncertain. The discharge rate of spindle receptors may be greatly increased by fusimotor action even at constant muscle length, allowing the existence of non-unique relations between length and discharge rate. Since isometric muscular contraction is accompanied by increased fusimotor activation (3), the occurrence of errors of perception of joint position under these conditions would support a significant spindle receptor contribution to positional sensation.

We examined the relation between muscle force and perceived steady-state joint position, using the proximal I-P joint of the right index finger in seven subjects. The finger was attached to an electromagnetic puller, capable of providing 20° of angular deflection under either positional or force servo-control. Perceived finger position was reproduced by active positioning of the left index finger, symmetrically mounted and attached to a length transducer. Measured variables were joint rotation on each side, and force output on the right side. Vision was excluded.

When the right index was moved passively, with constant speed, subjects were able to reproduce its final position with considerable accuracy. However, when subjects were required to produce an isometric contraction on achieving the final position, gross errors of perception occurred. These positional errors were directly proportional to the force being exerted by the subject, in a relation of the form: positional error = AF + C; F is force (g), A,C = constants. The mean value for A is .015°/g with a range of .007°/g to .023°/g and the value of C falls in the proximity of the actual displacement, as expected. When passive positioning of the left finger was reproduced by the right, working against isotonic or spring-like loads, no consistent errors of perception appeared.

The occurrence of marked positional errors with isometric contraction is evidence supporting a major contribution by spindle receptors to joint position sense, since their discharge rates increase approximately in proportion to the amount of force exerted (3). Limited human data (3), however, show that under isotonic conditions, there may be less force-dependent variation in spindle receptor discharge rate, perhaps accounting for the greater positional accuracy. Contributions from tendon organs or capsular receptors cannot of course be excluded by our findings.

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1532 BEHAVIORAL MODULATION OF TRIGEMINAL BRAIN STEM NEURONAL ACTIVITY IN AWAKE RHESUS MONKEY. R.Dubner, R.L.Hayes, S.L.Berg*, and T.P. Medlin*. Neurobiol. & Anesth. Br., NIDR, NIH, Bethesda, MD 20014

Unit activity was recorded in trigeminal nucleus caudalis and subjacent lateral reticular formation in monkeys trained in a reaction time task to discriminate warming and noxious heat stimuli applied to the face (Hayes et al., Neurosci. Absts., 1977). Neuronal activity was correlated with the following behavioral events: 1) panel light onset indicating that a new trial could be initiated; 2) panel press initiating onset of innocuous (37°-43°C) and noxious (45°-49°C) thermal stimuli; 3) termination of thermal stimuli, and 4) panel release. Three classes of neurons have been studied. Receptive fields were limited to the ipsilateral face and often were confined to the trigeminal maxillary division. Nociceptive neurons exhibited responses correlated with onset and termination of thermal stimuli. Thresholds often were below 40°C, but stimulus-response functions were positively-accelerating in the noxious heat range. A preceding noxious heat stimulus suppressed thermal responses. Behaviorally non-relevant thermal stimuli evoked longer latency responses which sometimes were reduced in magnitude, whereas responses to particularly relevant stimuli were potentiated. Phasic polysensory neurons had flatter stimulus-response functions than nociceptive neurons and responses also were suppressed by preceding noxious heat stimuli. They had phasic response components which correlated with panel light onset and panel press. Behaviorally non-relevant thermal stimuli evoked weaker responses in the innocuous range; responses in the noxious range were delayed in onset but of similar magnitude. Thus, stimulus-response functions produced by non-relevant stimuli were similar in shape to those of nociceptive neurons. Tonic polysensory neurons exhibited maintained discharges throughout the panel press period which ceased when the monkey escaped noxious thermal stimuli or detected the termination of innocuous warming stimuli. Response magnitude sometimes increased during panel press and often high-frequency discharges preceded panel release. Unit firing was enhanced by behavioral relevance of thermal stimuli and the appropriateness of the motor response. Response magnitude was independent of thermal stimulus intensity except for increased responses to 47° and 49°C noxious heat stimuli. Preceding noxious heat stimuli did not suppress thermal responses. Panel light onset produced either an increase or a decrease in activity. These data suggest that (1) nociceptive neurons provide information about the intensity of noxious heat stimuli, (2) phasic polysensory neurons signal the presence of behaviorally significant stimuli, and (3) tonic polysensory neurons are associated with the execution of motor behaviors evoked by salient sensory stimuli.

1533 LONG SPINAL PATHWAYS MODIFYING FORELIMB MECHANOSENSORY NEURONS. R. W. Dykes and D. G. Tanji*. Dept. Physiology and Biophysics, Dalhousie University, Halifax, N.S., B3H 4H7

In decerbrate cats, glass-coated tungsten microelectrodes were driven into the cervical spinal cord. Electrical stimuli were applied to the ipsilateral and contralateral sciatic nerves during a search for units that responded to natural stimulation of the forelimb. The sciatic nerves were left in continuity so that hindlimb receptive fields could be identified. Afferent fibers and postsynaptic cells were identified by standard criteria. 50 μ thionin-stained sections of the spinal cord were used to locate electrolytic lesions placed at the end of electrode tracks and to reconstruct the penetrations. It was observed that some postsynaptic units of the cervical spinal cord received excitatory and inhibitory influences following sciatic nerve stimulation. These long spinal effects from the lower limbs were produced by large as well as small fibers. Some of the units studied responded to low threshold cutaneous stimuli on the lower limbs. Others responded to noxious cutaneous stimuli. Several neurons were observed with inhibitory receptive fields on the forelimb which could be excited by hindlimb stimulation. Others displayed excitation from the contralateral forelimb as well as from the hindlimbs. Both ipsilateral and contralateral effects were evident in the data obtained from hindlimb stimulation, however ipsilateral effects were more common. The latencies and temporal distribution of impulses resulting from hindlimb stimulation suggested that both large and small myelinated fibers converged on the same cervical neurons. Although modification of cervical motor neuron activity by sensory input from the lumbar spinal cord has been reported previously, long spinal pathways which modify cervical somatosensory neurons have not been reported. These observations suggest that integration of long spinal reflexes may occur by alterations in afferent information as well as by modification of the final common path. Further, if supraspinal influences can also modify these same neurons they have properties comparable to the α-motor neurons of the ventral horn.

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1534 THE ORGANIZATION OF TRIGEMINO-THALAMIC NEURONS AS DETERMINED BY HORSERADISH PEROXIDASE RETROGRADE LABELLING. Takanori Fukushima*, Jack D. Grabow and Frederick W. L. Kerr. Mayo Foundation, Rochester, MN 55901.

Following injection of 30% horseradish peroxidase (Sigma VI) into the n.V.P.M. of 20 rats in doses ranging from 0.1 to 1.6 µl the distribution of labelled neurons in the trigeminal nuclear complex was determined using the diaminobenzidene method and compared with the distribution in other nuclei.

The organization of trigeminal projection neurons was much more complex than anticipated. In the main sensory nucleus and in n. oralis, the great majority of medium sized and many small neurons were labelled, while the largest neurons never contained H.R.P. In subnucleus interpolaris at the level of the facial nucleus, only a few small neurons were labelled, whereas in the caudal part the large stellate and polygonal neurons were strongly labelled together with a moderate number of small neurons.

Subnucleus caudalis showed yet another distinctive pattern: marginal neurons were frequently labelled, especially at the dorsomedial aspect of the nucleus. Considerable numbers of medium sized and small neurons in the s. gelatinosa were labelled, but none were marked in the nucleus proprius.

These highly distinctive and changing neuronal patterns correlate well with the original subdivision of the nucleus by Olszewski '50. The physiological role of the different nuclear groups is inferred from the similarity of patterns in main sensory and oralis to lemniscal nuclei, the non labelling of rostral interpolaris with projection to the cerebellum and of subnucleus caudalis, to the dorsal horn of the spinal cord. Caudal interpolaris is difficult to categorize, but may be comparable to lamina V of the dorsal horn. (Supported by NS 5995 from the National Institutes of Health.)

1535 AFFERENT AND EFFERENT COMPONENTS OF THE FACIAL NERVE IN THE BULLFROG: AN EXPERIMENTAL STUDY USING COBALT IONTOPHORESIS. Peter M. Fuller, Department of Anatomy, University of Louisville, Health Sciences Center, Louisville, Kentucky 40201.

Using adult bullfrogs (Rana catesbeiana) cobalt chloride was applied to the cut end of the seventh cranial nerve in 25 animals to dtermine the afferent and efferent components of the nerve. Following the cobalt treatment (Fuller and Prior, '75) the brains were fixed, embedded in egg yolk and serial sections cut at 33 microns. Alternate sections were stained for either cobalt or with a Nissl stain. In addition, a series of frog brains were processed with the rapid Golgi-Cox method in an attempt to further delineate the areas of termination of the afferent fibers and the origin of the efferent fibers.

There are three distinct components to the facial nerve in the bullfrog. The first is the motor component which arrises from the motor nucleus, situated medial and slightly caudal to the trigeminal motor nucleus. The cells of the facial motor nucleus are large and fusiform in shape. The motor fibers pass laterally from the nucleus and exit in close proximity to the afferent vestibular fibers of the eighth nerve. The second component of the facial nerve is sensory, and projects almost entirely to fasciculus solitarius. The fibers enter the fasciculus solitarius and are confined to the dorsal and dorsal-lateral portion of solitarius. Followed caudally, the number of fibers gradually diminishes as they terminate on the nucleus of solitarius. Fibers can be traced caudally to the first spinal segment. There are also a limited number of sensory fibers which enter the descending spinal tract of V. The third component of the facial nerve is proprioceptive. The large cells of the mesencephalic nucleus of V, located in the rostral third of the tectum, fill as a result of the cobalt iontophoresis. The fibers pass around the lateral edge of the tectum and then descend toward the ventral surface of the brainstem. Continuing caudally, the fibers pass lateral to nucleus isthmi and the cerebellar nucleus, and then exit with the remaining fibers of the facial nerve.

The motor and sensory components of the facial nerve have been reported previously in a variety of vertebrates, however, the proprioceptive component of the facial nerve is an entirely new finding.

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1536 AFFERENTS TO BRAINSTEM NUCLEI (BRAINSTEM RAPHE, n. RETICULARIS PONTIS CAUDALIS AND n. GIGANTOCELLULARIS) IN THE RAT AS DEMONSTRATED BY MICROIONTOPHORETICALLY APPLIED HORSERADISH PEROXIDASE. Dorothy W. Gallager and Agu Pert. Adult Psychiatry Branch, NIMH and Dept. Psychiat., Yale U. Sch. Med., Bethesda, MD 20014.

Recently much attention has centered on the brainstem raphe nuclei (BR) (including the n. raphe magnus, n. raphe obscurus, and n. raphe pallidus) especially the raphe magnus as a possible relay center for mediating analgesia and inhibiting spinal pain transmission by opiates. However, at present only relatively massive degeneration studies have been used to define the afferent pathways to this region. The present study describes afferents of the brainstem nuclear groups including the BR and two adjacent nuclei, the n. gigantocellularis (nGC) and the n. reticularis pontis caudalis (nRPC) as determined by the horseradish peroxidase (HRP) retrograde transport method. HRP was applied microiontophoretically, resulting in a very localized (0.3 - 0.8 mm dia.), highly concentrated deposit of the enzyme (2.2 ±0.3 µg as determined in vitro). The most striking difference between the afferent projections to the BR and the two adjacent nuclei as determined by this method is that afferents to the BR originate principally from structures rostral to the pons. In contrast, afferents to the two adjacent nuclei (nGC and nRPC) appear to originate primarily within or caudal to the pons-medulla. Afferents to the BR include: lateral and dorsal mesencephalic central gray (excluding the dorsal raphe area), dorsal and ventral tegmentum, deep layers of the superior colliculus, medial vestibular nucleus (VIIIM), and a few cells from the ventromedial part of the prefrontal cortex. Afferents from the nGC include: dorsal and ventral tegmentum, deep layers of the superior colliculus, VIIIM, scattered afferents from the dorsal part of the mesencephalic central gray and throughout the lateral and ventral horn, and the dorsal horn deep to Rexed lamina V in the cervical spinal cord. Afferents from the nRPC include the dorsal and ventral tegmentum, VIIIM, n. pontis oralis, and deep layers of the superior colliculus. In addition, evidence for interconnections between all the adjacent reticular nuclei (BR, nGC and nRPC) was found.

Such afferent projections are compatible with the notion that the brainstem raphe nuclei may serve as a connection within the brainstem for a descending system while the nGC may be a brainstem terminus for a spinal ascending system.

1537 COMPARISON OF MECHANORESPONSIVE PROPERTIES OF FIRST AND SECOND-ORDER VIBRISSAE-ACTIVATED TRIGEMINAL NEURONS. John M. Gibson, Wen-Deo Tang*, and Wally Welker, Dept. of Neurophysiology, University of Wisconsin, Madison, Wisconsin 53706.

Different nuclei of the trigeminal complex are thought to exhibit distinct functional and projectional features. We are testing the hypothesis of subnuclear specialization by examining, with quantitative methods, single-unit stimulus-response (S-R) transactions in the rat's sensory vibrissae system. Having established a baseline of coding properties in the primary afferent population (Gibson, et al., Neurosci. Abstr., 1976), we began our study of second-order trigeminal neurons in Nucleus Oralis.

Vibrissae-activated single-unit responses were recorded from barbiturate-anesthetized albino rats. Mechanical stimuli consisted of quantitatively-controlled deflections of single mystacial vibrissae, using a standardized battery of waveforms in which stimulus parameters were varied systematically.

The S-R profiles of second-order units exhibited many similarities to those of first-order units: (1) A broad spectrum of properties was observed. (2) Thresholds (angular displacement and velocity) had a range approaching three orders of magnitude. (3) Adaptation rates were continuously distributed. (4) In response to a standard step deflection (approx. 7^{o}), about half the units fired for more than 200 msec. (i.e., were more "slowly-adapting"). (5) Adaptation rate varied with stimulus amplitude.

However, striking differences between first and second-order S-R profiles were noted: (1) Nearly half the second-order units had multiple-vibrissae receptive fields (half of which included a patch of fur), whereas no first-order unit responded to movement of more than one vibrissa. (2) The median angular displacement threshold of medullary units was about 0.2^{o}, one-fifth the first-order value; over three-fourths of the second-order units were more sensitive than the median first-order unit. (3) The median angular velocity threshold was about 3^{o}/sec., versus 100^{o}/sec. at first order; almost 90% of the second-order units had velocity thresholds below the first-order median.

By means of objective, quantitative methods, and without recourse to the usual dicotomous categorizations, we have defined differences between first and second-order S-R transactions which we infer to reflect selective information processing by one subnucleus of the trigeminal complex. Our findings also emphasize the necessity of appreciating the diverse spectrum of multi-dimensional features which characterize a population of mechanosensory neurons. Future studies will examine these relationships in other trigeminal nuclear regions.

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1538 EM STUDIES OF DEGENERATIVE CHANGES IN THE AXONAL ENDINGS OF PRIMARY TRIGEMINAL NEURONS AND IN POSTSYNAPTIC NEURONS IN LAYERS I, II AND III OF NUCLEUS CAUDALIS FOLLOWING TOOTH PULP EXTIRPATIONS. Stephen Gobel and Joan M. Binck*. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20014.

The pulp chambers of teeth are innervated by hundreds of primary trigeminal neurons with either small myelinated (Aδ) or unmyelinated (C) axons. These neurons respond to a variety of noxious and thermal stimuli. Extirpation of tooth pulps results in amputation of the receptors of these primary trigeminal neurons and the subsequent filling of the pulp chambers with an inert material precludes the possibility of their regeneration. In order to determine the consequences of this procedure, a series of experiments in adult cats was conducted in which the tooth pulps of all mandibular teeth on one side were removed and the pulp chambers filled with dental cement. After survival times of 14, 30 and 60 days, degenerative changes are found in the axons of primary trigeminal neurons in the spinal tract and in their axonal endings in layers I, II and III of nucleus caudalis. The sequence of degenerative changes in the primary axonal endings in layer I differs from that in the primary endings in layers II and III. The axoplasm in layer I endings blackens and the endings are rapidly engulfed by glial cells. Degenerating primary endings in layers II and III, on the other hand, are not engulfed by glial cells but degenerate in situ over a more protracted period of time. They lose almost all of their synaptic vesicles and shrink in size but their axoplasm does not blacken.

In addition, neurons in layers I, II and III which receive synaptic input from the primary neurons innervating tooth pulps also degenerate. Transynaptic degenerative changes include a loss of organelles in cell bodies and dendrites, formation of large cavities in dendrites, breakdown of neurotubules in dendrites, a blackening of dendritic cytoplasm and withdrawal of spine heads from the glomeruli.

These experiments demonstrate that layers I, II and III of nucleus caudalis are major sites of termination of primary trigeminal neurons innervating tooth pulps. It is possible that the massive disruption in the synaptic circuitry of trigeminal pain pathways resulting from the loss of primary input from either tooth pulp extirpation or tooth extraction may be an important factor in some orofacial neuralgias.

1539 AIRJET STIMULATION OF HAIR RECEPTIVE FIELD ELEMENTS. M.D. Goldfinger and V.E. Amassian. Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

During continuous airjet stimulation of cat forelimb hairs, a primary afferent axon recorded by a glass-insulated tungsten microelectrode in the cuneate fasciculus yields maintained activity which, except at brief interspike intervals, approximates a random process (Goldfinger & Amassian (1976) Neurosci. Abst. II:911).

Previous investigators have shown that the parent hair afferent axon may be activated by stimulation of any one of the innervated receptive field hairs. Using airjet stimulation, we compared the responses of the parent axon during whole field and single hair stimulation - other hairs in the receptive field being pressed down with masking tape. The mean discharge rates with single hair stimulation always were a fraction of those obtained during whole field stimulation and often drifted. The single hair evoked discharge pattern also approximated a random process: ie, except at very short intervals, the interspike intervals were exponentially distributed, the expectation density function was flat, and the joint interval scatter plot indicated independence of successive intervals.

Because of spike train instability with single hair stimulation, spike trains must be compared from the onset of the airjet. Under this constraint, separate stimulation of two individual hairs of the same receptive field yielded considerable differences in parent axonal discharge rates (e.g. 95. & 122./sec; 80. & 114./sec; 66. & 98./sec; 25. & 82./sec, where each pair is from a different parent axon). The corresponding ratios of single hair: whole field discharge rates were: 1:2.6, 1:2.0; 1:2.7, 1:1.9, 1:3.4, 1:2.3; 1:9.9, 1:3.0. However, the number of guard hairs innervated by a parent axon is 4-9 in rabbit ear (Miller & Weddell (1966) J. Physiol. 187:291-305) and 10-20 in cat hindlimb (Brown & Iggo (1967) J. Physiol. 193:707-732), implying a less than linear summation in the whole field response to airjet stimulation. Simultaneously stimulating two hairs yielded a higher discharge rate than that during stimulation of either separately, but the observed rate was less than, equaled, or exceeded the algebraic sum of the separate rates. Simultaneously stimulating two hairs yielded a discharge rate lower than that with whole field stimulation.

In conclusion, the maximum whole field response to airjet stimulation does not appear to be explicable by the activity of a single 'dominant' hair within the field, but rather suggests a non-linear summation, at the axonal branch points, of activity derived from a number of hairs.

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1540 THRESHOLD AND SUPRATHRESHOLD SENSATIONS PRODUCED BY ELECTRICAL TOOTHPULP STIMULI AND THEIR MODIFICATION BY FENTANYL, A NARCOTIC ANALGESIC. Richard H. Gracely*, Patricia A. McGrath*, Marc W. Heft* and Ronald Dubner (SPON: Charlene D. Jarvis). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20014.

Sensations produced by toothpulp stimulation were examined in a series of experiments in which subjects (1) determined sensory detection and pain thresholds at different stimulation frequencies, (2) scaled the sensory intensity and unpleasantness of suprathreshold noxious stimuli and (3) rated electrical stimuli before and after the administration of fentanyl, a short acting narcotic analgesic. Electrical stimuli were 1-sec trains of monopolar, monophasic, cathodal, 1-msec duration, constant current pulses delivered to the labial surface and incisal ledge of upper incisor teeth. All subjects reported detection thresholds that were significantly lower than pain thresholds at all frequencies studied (Method of Limits). Mean pain thresholds varied nonmonotonically with frequency, decreasing from 26 μA at 5 Hz to 20 μA at 100 Hz, and increasing to 25 μA at 500 Hz. Sensory detection thresholds did not change with frequency (mean=8 μA, 5-500 Hz).

Forty subjects scaled the intensity and unpleasantness of pain produced by toothpulp stimuli by choosing words from randomized lists of either sensory (i.e. weak, moderate, intense) or affective (i.e. annoying, unpleasant, distressing) verbal descriptors validated in a previous study (Gracely et al., Neuroscience Abstracts 2:937, 1976). In addition to 100 Hz electrical stimuli, the subjects also rated sensations produced by a natural noxious cold stimulus (ethyl chloride spray) applied to exposed dentin. Responses to the controlled electrical stimuli produced reliable psychophysical functions linear in log-log coordinates (r=.99). These functions predicted the sensory intensity and affective verbal descriptor responses made directly to the natural stimuli, based on direct matches (Method of Constant Stimuli) between the electrical and noxious cold stimuli. In a final experiment, 32 subjects rated 100 Hz electrical toothpulp stimuli between pain threshold and pain tolerance before and after the I.V. administration of 0.05 mg fentanyl. Fentanyl significantly reduced the magnitude of the sensory intensity verbal descriptor responses, while the affective verbal descriptor responses were unaltered.

These series of experiments show that (1) detectable toothpulp stimuli do not necessarily produce pain even at frequencies up to 500 Hz, (2) pain scales developed with electrical toothpulp stimuli have validity in the evaluation of natural noxious stimuli, and (3) a narcotic analgesic drug reduced the perceived sensory intensity of noxious toothpulp stimuli rather than altering the affective aspect of the pain experienced.

1541 SPATIAL SUMMATION IN THE THERMOSENSORY SYSTEMS OF THE HUMAN AND THE MONKEY. Joel D. Greenspan* and Dan R. Kenshalo, Dept. of Psychology, Florida State University, Tallahassee, Fla. 32306

Three human observers were tested for their ability to detect warm and cool stimuli applied to their left palm. Four areas of stimulation were used--7, 4, 2, and 1 cm^2. For each area, the observers' sensitivity to four intensities of warming and cooling were assessed using a yes-no signal detection procedure. In addition, one monkey with prior experience in the same paradigm was given identical tasks with the exception that the monkey was also tested with a 0.5 cm^2 area of stimulation.

The human data for both warming and cooling indicated some degree of spatial summation under all conditions. That is, as the area of the stimulus was increased, the stimulus intensity decreased in order to maintain the same degree of detectability. The function which relates stimulus area and intensity for a given level of detectability is described by the formula:

$$I = kA^b,$$

where A is the area and I is the intensity of the stimulation. This is the same type of function that has been obtained by using classical psychophysical methods. For warming stimuli, an increase in area was found to increase detectability more for readily detected intensities than for those intensities less readily detected. A similar phenomenon was not found when cool stimuli were used. A change in area of a cool stimulus produced the same relative effect regardless of the detectability of the stimulus initially.

The monkey area-intensity function can be described by the same formula as that for humans. However, while the humans showed spatial summation over areas up to 7 cm^2, spatial summation in the monkey appeared to be limited to less than 2 cm^2. Possible variations in innervation density of the palms of these species may account for this difference.

(This research was supported by Grants NSF GB-30610 & USPHS NS-02992)

1542 RESPONSES OF JOINT AFFERENT NEURONS TO CONTROLLED DEFORMATIONS OF ISOLATED JOINT CAPSULE. Peter Grigg Dept. of Physiology, University of Massachusetts Medical School, Worcester, Mass. 01605, USA.

Recordings were made of afferents in cat medial articular nerve, innervating a section of knee capsule which was surgically removed from the cat and studied in vitro. Responses of afferents were studied in relation to applied stresses and strains. The capsullar material consisted of a small (1.0 x 0.5 cm) sheet which could be loaded either along its long axis (in-plane loading) or normal to the sheet (transverse loading). Most afferents could not be activated with pure in-plane loading. Those which were activated had high (over 50g) thresholds. All afferents studied, however, were readily activated using a combination of in-plane and transverse loadings. Under these conditions, most afferents had low thresholds for activation (transverse loads of 5g or less) and when activated were slowly adapting. Discharge in afferents appeared to be related to a multi-dimensional stress state in capsullar material, since for most afferents studied, responses were not a unique function of either in-plane or transverse loads. Supported by NIH grant NS- 10783.

1543 A (^{14}C)-2-DEOXYGLUCOSE METABOLIC MAPPING STUDY OF THE RAT POSTEROMEDIAL BARREL SUBFIELD. Peter J. Hand, Richard R. Miselis, and Martin Reivich. Depts. Animal Biology and Neurol., Sch. Vet.Med. and Med. and Inst. Neurol. Sci., U. of Pa., Phila., PA 19104.

The relationship of mystacial vibrissae with the posteromedial barrel subfield (PMBSF) of the first somatic sensory cortex (SI) was studied in six adult rats using the recently developed (^{14}C)-2-deoxyglucose (2DG) metabolic mapping technique (Kennedy et al. Proc. Natnl. Acad. Sci., 73: 4230-4234, 1976). Following selective clipping, various combinations of vibrissae were hyperstimulated with a hand-held brush to evoke neural activity, increase metabolic activity, and thus uptake of 2DG in neurons of PMBSF of unanesthetized, restrained rats. After ten minutes stimulation, 50 uCi of 2DG was pulse injected intracardially via a chronic catheter and vibrissae stimulated an additional thirty minutes. Rats were sacrificed by an intravenous injection of saturated KCL, brains removed rapidly and frozen in a Freon-dry ice mixture, and neural tissue prepared according to Kennedy et al. Stroking of all five rows of mystacial vibrissae resulted in increased 2DG uptake in the five rows of barrels of contralateral PMBSF. Stimulation of vibrissal rows A,B,D,E densely labeled all rows, excepting C. Conversely, vibrissal row C stroking heavily labeled row C. When a single vibrissa (No. 3, row C) was stroked, a remarkably discrete oval-shaped column of dense labeling, 400-um in diameter was observed within barrel 3, row C. The column, although extending throughout cortical laminae I-VI, was densest within layer IV. The heaviest uptake appeared within the barrel core with less dense labeling in its "wall". All barrels surrounding No. 3, row C exhibited less dense 2DG uptake than adjacent cortical regions (a negative image outlining PMBSF). Stimulation of two vibrissae (Nos. 2 & 4, row C) resulted in two discrete columns of labeling within the appropriate barrels of PMBSF. In addition, certain brainstem and thalamic nuclei were examined for 2DG uptake. Dense labeling was observed in ipsilateral principal (PV) and spinal (N.tr.sp.V) trigeminal nuclear complexes and the contralateral thalamic ventrobasal complex (VB). In all cases, dense uptake was conspicuously absent from subnucleus interpolaris of N.tr.sp.V, but was present in subnuclei caudalis and oralis. When comparing the effects of total vibrissal and row C stroking, the areal extent of labeling was similar in N.tr.sp.V, but densities were not. In contrast, their densities were similar, but areal extents not in PV and VB. In conclusion, these findings demonstrate: 1) the relationship of mystacial vibrissae with the barrels of PMBSF, VB, PV and N.tr.sp.V, 2) the basic columnar organization of SI, and 3) the value of the 2DG technique in mapping somatosensory connectivity. (Supported by Grant NS06716).

1544 COMPARISON BETWEEN NEURONAL SAMPLES FROM PERICRUCIATE AND PRECORONAL CEREBRAL CORTEX OF CHLORALOSE- and BARBITURATE-ANESTHETIZED CATS. G.W. Harding*, R.M. Stogsdill*, and A.L. Towe (SPON: M.D. Mann). Dept. Physiol. & Biophys. (SJ-40), Univ. of Wash. Sch. of Med., Seattle, WA 98195.

Single neuron samples from the sigmoid gyri of chloralose-anesthetized cats differ from those of barbiturate-anesthetized cats primarily in having many more wide-field neurons. The fate of these neurons was studied by comparing samples from four cerebral sites in barbiturate cats with a reference sample from comparable sites in chloralose cats. Standard procedures were followed, with the exception that the entire experiment was automated—including single neuron isolation and tracking. Recording in forepaw cortex and using a supramaximal shock to the central footpad of the contralateral forepaw as a hunting stimulus, 435 excitable neurons were obtained. Sufficient information was obtained on 374 of these for use in this analysis. The average number of excitable neurons per electrode penetration at a given site serves as an index of overall excitability within that site. In comparison with chloralose, excitability under barbiturate was near normal in precoronal tissue (field 3b), but was markedly reduced in pericruciate tissue (field 4). The neurons were partitioned into sets: sa, sb, and m (small-, bilateral, and wide-field, respectively), and compared with the same sets in a reference chloralose sample of 3390 neurons. The table shows the proportions observed relative to those expected, by sets, at each recording site.

Set/Site	Precruciate	Postcruciate	Midsigmoid	Precoronal
sa	0.27	0.62	1.11	0.94
sb	0.17	0.42	2.67	2.88
m	0.005	0.03	0	0.96

Both the distributions in depth and the response properties of the sa and sb neurons under barbiturate were similar to those under chloralose (with somewhat shorter response latencies), rather than being an amalgum of sa, sb, and m neuron properties. Thus, the excitability of m neurons is nearly zero under barbiturate (because of sample size, the 0.96 value in precoronal tissue may be spurious); the m neurons are not converted into sa or sb neurons under barbiturate anesthesia. Further, though the excitability of sa neurons is the same, and that of sb neurons greater, in barbiturate than in chloralose for precoronal (field 3b) and midsigmoid (field 3a) tissue, it drops significantly in pericruciate (field 4) tissue, especially at the more anterior site. Evidently, barbiturate selectively depresses and/or chloralose enhances the projection pathway(s) into pericruciate tissue. (Supported by NS00396 and NS05136)

1545 DIFFERENTIAL EFFECTS OF A NARCOTIC AND A MINOR TRANQUILIZER ON DETECTION AND DISCRIMINATION OF THERMAL STIMULI BY RHESUS MONKEY. R.L. Hayes, R.D. Harris, P.J. Wolskee* and R. Dubner. Neurobiol. and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20014.

Two water-restricted rhesus monkeys were trained to detect the termination of innocuous heat pulses delivered via a feedback-controlled contact thermode (1 cm diam.) applied to the upper hairy lip. Adapted skin temperature was 35°C. A panel was illuminated to signal the beginning of each trial. Monkeys depressed the lighted panel to initiate innocuous heat pulses (39°, 41°, 43°C) of randomly varying durations (2-8 sec). Panel releases of less than 2 sec (reaction times or RTs) after the termination of these stimuli were rewarded by water (0.5 cc). Additional heat pulses of a constant duration (20 sec) were presented quasirandomly in which the temperature increase reached the noxious range (45°, 47°, 49°, 51°C). Release of the panel at the termination of noxious stimuli was not rewarded by water. However, early releases terminated heat pulses on these trials, thereby allowing the monkeys to escape noxious heat stimuli. Thus the task assessed RTs indicating the detection of the termination of innocuous heat stimuli or the discrimination of the onset of higher stimulus intensities shifting into the noxious range. The effects of both the narcotic, morphine sulfate, and diazepam, a minor tranquilizer and muscle relaxant, were assessed at intramuscular doses of 0.5, 0.75 and 1.0 mg/kg. The highest dose produced some behavioral toxicity with both drugs. Diazepam significantly increased the RTs for the detection of the termination of all innocuous stimuli at every dose tested. The effect was dose dependent but not related to the magnitude (ΔT) of the innocuous temperature shift, thus suggesting a non-specific, drug-produced increase in RTs. Morphine did not significantly affect RTs to innocuous stimuli at any ΔT. Analysis of drug effects on the discrimination of the onset of noxious stimuli was done using RTs corrected by each drug dose for the non-specific increase in RTs observed for innocuous ΔTs. Diazepam did not significantly affect RTs to the onset of any noxious stimuli. Morphine had a dose dependent effect on RTs to noxious stimuli. A 0.5 mg/kg dose reliably increased RTs to 45°C; 0.75 mg/kg increased RTs to 45° and 47°C; 1.0 mg/kg, in spite of some behavioral toxicity, increased RTs to 47°C. Thus, at the doses tested, morphine but not diazepam affected discrimination of the magnitude of temperature shifts into the noxious range. These results suggest that: (1) Drug-produced changes in emotionality are not sufficient to influence discrimination performance in this task since diazepam, a putative anxiety reducing agent in animals and humans, produced only non-specific effects, and (2) narcotics can affect a sensory-discriminative component of responses to noxious temperature shifts.

1546 MODULATION OF AFFERENT SIGNALS IN THE SOMATOSENSORY SYSTEM BY PRIOR INPUT. C.L. Hinman*, J.S. Kroin*, R.J. Sclabassi*, H.A. Risch* (SPON: J.S. Buchwald). Brain Res. Inst., UCLA, Los Angeles, CA, 90024.

Nonlinear interactions resulting from a series of peripheral stimulations with inter-stimulus intervals of less than 500 msec have been found to occur among responses in the somatosensory system of cat and man. A characterization of cat somatosensory responses to trains of interactive peripheral stimuli was accomplished by the mathematical method of functional power series analysis. A random train of impulses having an exponential distribution of inter-stimulus intervals was delivered to the superficial radial nerve. A set of system kernels was then calculated from responses evoked in the contralateral cuneate nucleus (DCN), in the nucleus ventralis posterolateralis (VPL), and at somatosensory S1 cortex in order to describe nonlinear interactions occurring at those locations. Theoretically, first-order kernels provide the characteristic response of the system to a unitary impulse, and second-order kernels approximate the nonlinearity in the response due to pairs of interacting impulses. Experimental results confirmed the theoretical predictions and also reflected the nonlinearity derived from 2-pulse recovery cycle experiments. Concerning the source of such nonlinear interactions in the somatosensory system, ablation of S1 cortex altered specific aspects of the kernels from both DCN and VPL, indicating that one possible method by which prior input normally modulates afferent signals at those loci is by the activation of cortical feedback loops at latencies of less than 5 msec and 15 msec, respectively. (Supported by USPHS Grants NS-02501, NS-11443, and by Grant 516 C from the National Multiple Sclerosis Society).

1547 *THE EFFECTS OF L-DOPA ON THE RESPONSIVENESS OF DORSAL HORN INTERNEURONS TO MECHANICAL SKIN STIMULATION.* *Charles J. Hodge and Charles Woods**.

Single cell recordings were made from 50 lumbar dorsal horn interneurons in both intact and spinal cats. The responses of the cells to skin or hair displacement were measured before and after the intravenous injection of L-DOPA in amounts ranging from 5 to 100 mgm/kgm. Following low doses of L-DOPA (less than 25 mgm/kgm) an increase in responsiveness was consistently found. This was manifest by an increase in the average number of spikes per stimulus, frequent increases in receptive field size and at times an increase in spontaneous activity. Some cells showed a change in the modalities of skin stimulation to which they would respond. Neither the sensory characteristics of the cell in the control state nor its laminar location, as determined by marking with fast green, had a significant influence on the changes in responsiveness seen after L-DOPA. Larger doses of L-DOPA caused significant inhibition with characteristics suggesting a separate mechanism, possibly serotonin release in the spinal cord. Results were similar in spinal and intact animals. The results support the hypothesis that L-DOPA causes sensory alterations in part, at least, by affecting sensory transmission at the segmental level.

1548 TACTILE DISCRIMINATION DURING NEGLECT FOLLOWING UNILATERAL LESIONS IN LATERAL HYPOTHALAMUS OF RATS. Lee Hoyman*, G. Daniel Weese*, and Gabriel P. Frommer. Dept. Psychol., Indiana U., Bloomington, IN 47401

Marshall et al. (Science, 1971, 174, 523-525; J. Comp. Physiol. Psychol., 1974, 86, 375-395) showed that rats fail to orient towards tactile stimuli contralateral to unilateral lesions in lateral hypothalamus (LH). To determine if such neglect is associated with deficits in detecting tactile stimuli, we compared orienting responses with learned responses guided by lateralized somatic discriminative stimuli (S_d). We found that rats that failed to orient to tactile stimuli contralateral to the LH lesion could still use such stimuli as S_d to guide learned responses, but they could not make responses contralateral to it.

Female hooded rats were anesthetized, blinded, and implanted with a lesioning electrode in LH, verified terminally from frozen sections. They were trained in a small, clear plastic restraining box with openings on either side through which tactile S_d ($\geq$ ½g) could be delivered. Three 1.3 cm dia. holes, one in the front wall and one each near the front of the side walls, were equipped with photocells to detect nose-poke approach responses which required only head movements to make. Correct approaches were rewarded with access to sugar water. Each rat mastered (96% correct) one of three tasks: 1) approach whichever side hole was on the same side as S_d; 2) approach whichever side hole was on the side opposite S_d; 3) approach front hole whenever either side was touched (Go No Go). The LH lesion was then made electrolytically through the implanted electrode. Sixteen of 22 rats showed contralateral neglect for at least two days. Those trained on Task 1 or 2 showed severe deficits, but only on trials on which responses contralateral to the lesions would have been correct. Task 2 was critical: all six rats responded correctly to S_d contralateral to the LH lesion (because responses were directed ipsilateral to the lesion), but made many errors of commission to S_d ipsilateral to the lesion but few of omission (showing they detected the stimuli but turned incorrectly to the ipsilateral hole). The four rats in Task 3 (Go No Go) showed a small, transient increase in false alarms and in latency to respond to stimuli ipsilateral to the lesion, perhaps due to exaggerated competing orienting responses to them.

These data show that rats exhibiting neglect on the orienting response measure could detect somatic stimuli contralateral to LH lesions. The data suggest that neglect following LH lesions is based on the failure of tactile stimuli to acquire control of responses contralateral to the lesion. (Supported by PHS grants MH 26973 and S05 RR 7031)

1549 FUNCTION AND MORPHOLOGY OF HORSERADISH PEROXIDASE IDENTIFIED CELLS IN RAT SmI "BARREL" CORTEX. Von Ayre Jennings* and John R. Bartlett. Center for Brain Research, University of Rochester, Rochester, New York 14642.

Under urethane anesthesia over 200 units located in the "barrel region" of rat SmI cortex have thus far been classified in terms of their responses to controlled manipulations of contralateral vibrissae and a number of these have been identified by ionophoretically injecting horseradish peroxidase (HRP) intracellularly. In many, but not all cases, a unit responded to manipulation of more than one vibrissa. Commonly seen were: 1) "on-phasic" units giving a burst of discharges at only the onset of vibrissa movement, 2) "on-off phasic" units similar to 1) but discharging at both the onset and end of movement, 3) "tonic" units whose discharge was maintained at an elevated level during maintained displacement of a vibrissa, 4) "tap" units responding only to very fast (>150°/sec) vibrissa movement and 5) "whisking" units whose discharge rate increased during the spontaneous vibrissae movements seen during light but sufficient anesthesia. In some cases of the latter, the unit's activity was inhibited if the moving vibrissa contacted a stationary object. Where more than one vibrissa affected a unit, three interactive effects were seen; summation, where movement of two vibrissae was more effective than one (1+1>1), a lack of summation where the effect of two vibrissae equaled that of either one (1+1=1) and "absolute" summation where a unit discharged only if both vibrissae were moved (0+0=1). Thus far no cases of inhibition have been seen.

Recording and HRP injection is via 0.1-0.5μ, 300-500 megohm pipettes whose tips are beveled using a unique piezo-electric device. The HRP is dissolved in a Tris-HCl buffered KCl solution. Cells from the above categories have been identified as pyramidal and both spiney and smooth stellate types. Although about 10% of the units studied are now being successfully labeled with HRP, the total number so far identified is still too low to allow a meaningful comparison of morphology with response classification. (Supported by NIMH Grant #MH 05456).

1550 TACTILE SPATIAL DISCRIMINATION ON THE PALM: PSYCHOPHYSICS AND PERIPHERAL NEURAL CODING. K.O. Johnson, J.R. Phillips*, and I. Darian-Smith. Dept. Physiol., Univ. Melbourne, Melbourne, Victoria. Australia

The sense of touch in the glabrous skin of the hand, like the visual sense, is primarily concerned with spatial information. A further similarity to visual function is that this tactile sensation is based on three receptor types each having different sensitivity curves across the spectrum of mechanical frequencies. The aim of the work reported here was to isolate and analyze the characteristics of spatial discrimination subserved by one of these receptor populations, the slowly adapting Merkel receptors. The following data describe studies in which we have tried to define the human capacity to discriminate stationary spatial patterns and in which we have mapped the spatial profiles of peripheral neural activity evoked by various stationary patterns in M. nemestrina.

In our psychophysical studies we have attempted to define the tactual acuity for stationary stimuli in a way that will allow us to place constraints on the possible mechanisms of spatial processing. We have used three types of spatial stimuli:

1. Modified Landolt C test. Subjects were asked to discriminate between a radius with a gap and one without. The minimum gap that was discriminated reliably (75% correct) was 0.7 mm.
2. Grating orientation test. Two square wave gratings were applied successively to a subject's finger tip, the first with the gratings parallel to the finger and the second parallel or orthogonal to the first. Gaps as small as 0.8 mm allowed reliable discrimination (75% correct).
3. Letter recognition. Subjects were asked to name letters of the alphabet when they were applied to the finger tip. After brief exposure to the task subjects readily recognized letters that were 8.0x6.0 mm; preliminary experiments suggest that the threshold is lower.

The measures of acuity reported here relate to one another in a way similar to the corresponding measures of visual acuity.

The spatial profile of discharge in the slowly adapting fiber population reconstructed from studies of single fibers can be characterized by four observations: (a) the dynamic component of the response is much more widespread than the static component, (b) the static component decays by half within 0.5 mm of an edge or a point, (c) slowly adapting receptors are primarily sensitive to shear stresses in the skin and (d) the response properties are much less variable than those of quickly adapting receptors or Pacinian corpuscles. The shear sensitivity results in edge enhancement in the discharge profile; the discharge at the edge of a flat stimulus is three times that in the flat region.

1551 THE ACTION OF IONTOPHORETICALLY APPLIED SEROTONIN ON SPINOTHALAMIC TRACT NEURONS IN THE PRIMATE. D.R. Kenshalo, Jr.,* L.M. Jordan, R.F. Martin, L.H. Haber, and W.D. Willis. The Marine Biomedical Institute and Depts. of Physiology & Biophysics and Anatomy, Univ. Texas Med. Br., Galveston, TX 77550 and Dept. of Physiology, Univ. of Manitoba, Winnipeg, Canada R3E OW3.

Stimulation in the region of the raphe magnus nucleus results in an inhibition of activity of spinothalamic tract neurons. The cells in the raphe nuclei are known to contain serotonin, which has a depressant action on many dorsal horn interneurons in the cat. If serotonin has a depressant action on primate spinothalamic tract neurons, this would suggest a direct inhibitory pathway from the brainstem onto neurons believed to signal pain.

Spinothalamic tract neurons were identified in the lumbosacral and sacral spinal cord of monkeys. Iontophoretic application of serotonin onto 31 cells resulted in a depression of activity in 19 of these. The depression of activity was progressive rather than immediate, which suggested a buildup of the drug was necessary. In contrast, when current effects were encountered, the effects were always of immediate onset. The depression of activity continued after serotonin application was terminated, whereas current effects terminated immediately. In five cells, pulses of glutamate were interacted with the depressant action of serotonin. Glutamate produced an excitation which was partially blocked by application of serotonin in all cases. A separate population of spinothalamic tract cells, which responded to receptors in deep tissue but not to cutaneous receptors, were powerfully excited by serotonin. A total of five cells of this type were encountered, and in each case the excitation was slow of onset and lasted well beyond the application of the drug.

In those cases in which a depressant action of serotonin on units responding to cutaneous stimuli could not be demonstrated, it was possible that the microelectrode array was too far away from the cell to allow a buildup of an effective concentration of the drug. The interference with the action of glutamate excitation by serotonin suggests that serotonin has a direct effect on the postsynaptic membrane of these spinothalamic tract neurons. If this is the case, the possibility that raphe-spinal cells form a direct inhibitory path to spinothalamic cells becomes a reasonable hypothesis.

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1552 SOMATOTOPIC ORGANIZATION OF THE BRACHIAL CORD OF CAT. H. Richard Koerber*, and Paul B. Brown. (SPON: W.E. Gladfelter). Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, West Virginia 26506.

Spinal cord segments C_4-T_2, the segments receiving forelimb cutaneous input, were characterized in adult cats of both sexes by recording from single units with stainless steel microelectrodes.

The dorsal horn dermatomes correspond closely to the dermatomes of the corresponding dorsal roots, with the exceptions that C_4 and C_5 dorsal horn dermatomes contain input from the forelimbs which is not part of their dorsal root inputs, and dorsal horn dermatomes are larger than the dermatomes of their dorsal roots.

Cells with receptive field centers on the proximal limb were generally located in the lateral dorsal horn and cells with distal receptive fields tended to be located more medially.

The dermatomal trajectory courses from the shoulder, down the anterior surface of the forelimb, across the ventral forepaw and up the posterior surface of the limb onto the thorax, with shifting overlap, and little receptive field overlap between the preaxial and postaxial limb.

As is the case is other nuclear regions of the CNS, the relative area devoted to projections from the forepaw is disproportionately large relative to the area devoted to cutaneous regions of similar size which are located more proximally on the limb. The areas of greatest representation density are the ventral forepaw and both the medial and lateral aspects of the wrist. There is a secondary focus of representation density at shoulder and axilla, corresponding to the secondary focus at the perineum in the lumbosacral maps of the hindlimb. Receptive fields were larger and more elongated on the leg than on the foot.

We conclude that the organization of forelimb dorsal horn projections to the cervical enlargement in the cat spinal cord obeys similar organizational rules to those previously observed in the projections of hindlimbs to the lumbosacral enlargement.

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1553 PRIMATE PARESTHESIAS: SPECIFIC AND NONSPECIFIC ASCENDING SPINAL MECHANISMS. M. Levitt and J. H. Levitt, Dept. Physiol. Pharmacol., Bowman Gray Sch. Med., Winston-Salem, N.C. 27103

We have described objective evidence of disturbing lumbosacral paresthesias in monkeys consequent to thoracic contralateral anterolateral cordotomy or hemisection (Fed. Proc. 36:538, 1977). The laterality does not suggest release from descending inhibition as a cause. A chronic irritative focus at the surgical site was deemed unlikely, since paresthesias were unchanged after subsequent surgical repetition, 4 segments rostrally. Paresthesias of cordotomy were similar to those resulting from total limb de-afferentation, or an upper lumbar syrinx (African Green monkey); suggesting a common, though unknown, neural mechanism. Others have concluded that interruption of the "spinothalamic system" is a primary cause of central pain in humans (Cassinari & Pagni, Central Pain, 1969). A remaining nonspecific ascending system was postulated to be essential. The paresthesias in monkeys after thoracic cordotomy were found to be independent of peripheral input (L_4-S_2 roots) from the affected dermatomes, or (with supramaximal cordotomy) of conduction via other paucisynaptic sensory tracts; however, they appeared to be dependent upon diffusely located homolateral mechanisms. Indeed, thoracic AL cordotomy or hemisection did not prevent the occurrence of bilateral paresthesias after second-stage bilateral L_4-S_2 posterior rhizotomies; or after second-stage hemisection on the other side and two segments away. These findings suggested that the paresthesias of cordotomy or rhizotomy might have been sustained by bilaterally, diffuse, polysynaptic spinal mechanisms. The paresthesias resulting from thoracic cordotomy were greatly attenuated, but not abolished after complete L_1-$_3$ spinal transection, 3-12 months later in 4 monkeys. Post-transection survival times ranged from 14-54 days, during which similar signs of paresthesias were detected in the leg contralateral to the cordotomy or hemisection. In one of these cases, a unilateral lumbar sympathectomy was then performed, which failed to stop the paresthesias. These findings suggest that the paresthesias of thoracic AL cordotomy are caused by the generation of chronic abnormal discharges of a somatotopically organized neuronal pool at a brain (diencephalic?) level; and the manifestation of the paresthesias is dependent upon central excitability states which are determined by nonspecific neural influences acting upon the discharging pool, and in part arising at caudal spinal levels. The report of C.N. Liu (Anat. Rec. 178:403, 1974) suggested that the ascending nonspecific influences can be modified by prior bulbar pyramidotomy. (Supported by USPHS Grant NS 11921).

1554 CENTRAL TERMINATION OF IDENTIFIED CUTANEOUS AFFERENT UNITS WITH FINE MYELINATED FIBERS. A.R. Light* and E.R. Perl. Dept. Physiol., Univ. North Carolina, Chapel Hill, NC 27514.

In anesthesized cats and macaque monkeys, the spinal cord distribution and synaptic termination of single, physiologically characterized primary afferent neurons was determined. The emphasis was on slowly-conducting myelinated fibers, but elements with conduction velocities ranging from 7.5 to over 70 M/sec were studied so that the projection pattern of the fine myelinated fibers could be compared with that of the coarse myelinated fibers. Unitary recordings were made from intact dorsal roots using micropipette electrodes filled with horseradish peroxidase (HRP). After the functional characteristics of a unit were determined by examining its response to natural stimulation of the skin, HRP was iontophoretically introduced into the fiber by a modification of the technique reported by Light and Durkovic (J.Exp. Neurol.53: 857, 1976). The single fibers stained by the reaction product of HRP could be traced into the spinal cord for several mm with main branches, extensive arborization of fine collaterals, and presumed synaptic thickenings clearly visible. The three dimensional distribution of thses fibers was reconstructed using an interactive, computer-based graphics system. Fourteen (14) neurons identified as high threshold mechanoreceptors, i.e. mechanical nociceptors (HTM), which had conduction velocities between 7.5 and 40 M/sec were found to have many synaptic enlargements in the dorsal horn marginal zone (lamina 1 of cat), and a few enlargements in the substantia gelatinosa (lamina 2 of cat). In addition, at least the more rapidly conducting and more sensitive of the HTM fibers also had a branch with synaptic enlargements in the medial and central portions of the nucleus proprius (lamina 5 of cat). D-hair afferent units (conducting between 11 and 20 M/sec) had a different termination pattern with a few synaptic enlargements in the marginal zone and a predominant distribution of terminals in the nucleus proprius (laminae 4 and 5 of cat). Other myelinated fiber receptors (G-1 and G-2 hair follicle, Type I and II slowly adapting cutaneous, field, and Group Ia and II muscle) each exhibited a characteristic pattern of terminations. However, none of these primary afferent types had en passant or terminal enlargements in the marginal or SGR zones. These data suggest that activation of marginal zone neurons by HTM reported for cat (Christensen and Perl, J. Neurophysiol. 29:293, 1970) and for monkey (Kumazawa and Perl, in Sensory Functions of the Skin. Y. Zotterman, Ed., Pergamon, Oxford, 1976) is probably monosynaptic and similar direct connections exist to certain neurons in the neck of the dorsal horn.

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1555 THE UNIT ACTIVITY OF SOMATOSENSORY AFFERENTS FROM CAT HINDLIMB DURING NORMAL WALKING. Gerald E. Loeb and Jacques Duysens. Laboratory of Neural Control, NINCDS, NIH, Bethesda, MD 20014.

The activity of individual somatosensory afferents from cat hindlimb during normal walking has been studied using a new method of implanting "floating" wire microelectrodes in the dorsal root ganglia (Neurosci. Abst. #782, 1976). 67 units from 11 cats have been so studied and subsequently characterized by physiological tests under anesthesia. The entire range of myelinated and unmyelinated afferents (0.8 to 108 m/sec) appears to be sampled by this technique, yielding the following preliminary observations:

Of 18 hair receptors (40 to 94 m/sec), almost all had highly repeatable firing patterns during walking even when their fields were not contacted by objects (e.g. on dorsum of foot or lateral hip).

14 light touch receptors (27 to 87 m/sec) were generally inactive unless their receptive fields contacted an external object. Skin stretch sensitivity appeared to modulate or initiate activity in some but not all of 4 stretch sensitive units.

11 spindle primaries (70 to 105 m/sec) and 4 spindle secondaries (43 to 57 m/sec) generally responded to passive stretch and to an apparently variable alpha-gamma coactivation. Some units had only one activity peak during passive stretch. Others had an additional peak during active muscle contraction. This latter activity could reach frequencies above that of the passive stretch peak but usually showed a pause during maximum shortening rate of the muscle. In our scattered sample, the only systematic difference between primaries and secondaries appeared to be higher peak firing rate in some but not all primary endings.

4 Golgi tendon organs had activity closely correlated with EMG activity and, presumably, active tension of their muscles, with very little passive stretch response during gait or manipulation.

5 knee joint receptors (46 to 75 m/sec) had very complex and variable activity patterns not simply related to joint angle, and possibly influenced by joint loading and/or capsular muscle tension. Units with similar responses to manipulation could have very different activity during walking.

There were 10 units of undetermined modality, often with complex bursting and/or long periodic cycles of spontaneous activity usually not modulated by gait. One unit was modulated by blood pressure changes, but others have been unresponsive to a variety of thermal, noxious, and visceral stimuli, and may represent the poorly understood classes of free endings and/or sympathetic afferents.

1556 INHIBITION IN SUBMODALITY CHANNELS INFLUENCING SI CORTICAL MECHANORECEPTIVE NEURONS. J. Martin* and W.A. Spencer* (SPON: K.R. Weiss). Div. Neurobiol., Dept. Physiol., Coll. Phys. and Surg., Columbia U., New York, N.Y. 10032

Afferent inhibition of unit responses evoked in primary somatosensory cortex is believed to participate in spatial and temporal contrast mechanisms operating within a given submodality channel of the primary projection pathway. But the identification, with stimuli selective for different peripheral mechanoreceptors, of central segregation of mechanoreceptive channels suggests an additional function: inhibition might arbitrate competing claims of different mechanoreceptive submodalities by being distributed from one submodality channel onto another.

In the present study single unit discharge patterns of quickly adapting SI cortical neurons in cats lightly anesthetized with Na Thiopental were examined to assess inhibition generated by sinusoidal mechanical stimuli delivered to their peripheral receptive fields. Cells responding to displacement of hairs were found to be tuned to low frequency skin indentations (flutter). Cortical units believed to be driven by Pacinian afferents (PC cells) were shown to be tuned to higher frequency stimuli (vibration) and to have receptive fields located deep to the skin. Anesthetic conditions allowed surgical decoupling of stimuli to skin and deeper tissue to achieve optimal selectivity. Several experimental conditions were examined using three different stimuli to various loci on the cat contralateral forelimb. Hair and PC cell test responses to effective single sinusoids at receptive field centers were paired with steady skin indentation, flutter or vibration conditioning stimuli delivered to nearby loci, and usually presented 15 ms. before the test stimulus.

Suppression of the test evoked single unit response of quickly adapting cortical cells was regularly observed when the conditioning stimulus was limited to the submodality of the cell under investigation. Little or no suppression was observed when the conditioning stimulus selectively activated submodality channels other than that driving the cell. Thus, a 20 Hz flutter stimulus strongly inhibited the test response of hair cells but not PC cells, and a 200 Hz vibration had exactly the opposite effect. A steady skin indentation inhibited the test response of some PC cells to a limited extent; yet, under no circumstance so far examined did this form of stimulation inhibit the test response of hair cells.

Our data thus tend to minimize the effects of cross-submodality inhibition. They accord with recent psychophysical inferences (Zambelli et al., 1976) that afferent inhibition is primarily important in shaping information concerning stimulus locus and distribution within a given submodality channel.

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1557 PROJECTIONS FROM THE SPINAL CORD AND THE GRACILE, CUNEATE, TRIGEMINAL AND LATERAL CERVICAL NUCLEI TO THE POSTERIOR GROUP OF NUCLEI IN THE THALAMUS OF THE CAT. D. C. Mash* and K. J. Berkley. (SPON: Judith Tunkl Blumsack). Dept. Psychol., Fla. St. Univ., Tallahassee, FL 32306.

The distribution within the posterior group of terminals from these various afferent sources was studied using a differential labeling strategy in which both autoradiographic and degeneration tracing methods were employed to label two different afferent pathways simultaneously in the same cat. This strategy allows one to make direct comparisons between the terminal distributions of the two afferent pathways under investigation.

It was found that there was considerable overlap between the projections of all of these pathways throughout most of their combined zone of termination in the medial division of the posterior group (POm). Within this highly convergent termination zone, however, certain differences were observed. Terminals from the trigeminal n. tended to be more heavily distributed further caudally and medially within POm than those from the n. cuneatus, while n. cuneatus terminals in turn tended to be more heavily distributed further medially and caudally than those from the n. gracilis. Although the projections from the lateral cervical n. extended throughout most of the region in POm that received gracile, cuneate and trigeminal fibers, its terminals were more heavily distributed laterally within this zone. Projections from the spinothalamic tract, on the other hand, tended to be more concentrated in the medial portions of this zone. In individual experiments, it was often observed that fibers from each of the two pathways under investigation did not necessarily appear to terminate on precisely the same group of cells; they appeared instead to terminate on neighboring cells within the same small region.

These results provide an anatomical basis for the results of electrophysiological experiments which show that the response properties of cells in the somatic sensory portions of the posterior group vary over a wide range and that cells with different response properties tend to be spatially commingled rather than separated in this region.

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1558 THE RELATIONSHIP OF CYTOARCHITECTURE AND DENDRITIC MORPHOLOGY TO AFFERENT INPUT WITHIN THE THALAMIC VENTRAL TIER NUCLEI OF THE RAT. J.P. McAllister*, D.M. Fekete*, J. Wells and D.K. Ryugo (SPON: J.S. Schwaber). Dept. Anat., Univ. Vermont, Burlington, VT 05401.

The present report addresses the relationship between the pattern and type of afferent input to the thalamic ventral tier nuclei and the unique morphological properties of the corresponding recipient target tissue. In Fink-Heimer preparations, lesions of the dorsal column nuclei (DCN) produce heavy contralateral degeneration in the lateral and anterolateral portions of the ventral posterior nucleus (VPL) as well as sparse degeneration in the central intralaminar nucleus (CIN). The primary terminal field in VPL contains fusiform neurons whose somata and tufted dendrites form a series of lamellae oriented parallel to the incoming lemniscal fibers. The secondary terminal field is marked by fine degenerating fibers and large neurons with multipolar, unbranched radiating dendrites. Spinal cord (SC) hemisections produce a primary terminal field in the ipsilateral rostral VPL, overlapping slightly with the most anterolateral region of DCN projections, and a secondary bilateral field in CIN. These degeneration patterns essentially confirm the observations of Lund and Webster (JCN, 1967a,b). The deep nuclei of the cerebellum (Cb) project contralaterally to the ventral medial (VM), ventral lateral (VL), central lateral (CL) and paracentral (Pc) nuclei, as well as to CIN. Some of the degenerating fibers recross the midline in the thalamic commissure to terminate in the central medial (CM) and Pc nuclei. The primary targets of Cb projections, VM and VL, are characterized by a mixed population of round, fusiform and multipolar neurons whose moderately branched dendrites are distinct from those in the adjacent regions. These Cb projections resemble those of the opossum (Walsh and Ebner, JCN, 1973).

These observations reveal that projections from SC, DCN and Cb exhibit a dual termination pattern within the dorsal thalamus. Each of these thalamic afferent systems projects to a primary target receiving a segregated input, and to a secondary target receiving multiple inputs. Finally, these target areas each have morphological characteristics which tend to complement the type and pattern of afferent input.

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1559 CHANGES IN THE PARAMETERS OF SIGNAL DETECTION THEORY PRODUCED BY SUGGESTION AND TRANSCUTANEOUS ELECTRICAL STIMULATION. Douglas B. McCreery* and James R. Bloedel. Dept. Neurosurg., Med. Sch. Univ. Minnesota, Minneapolis, MN 55455.

Experiments were performed to assess the effect of transcutaneous electrical stimulation on the perception of non-noxious and noxious thermal stimuli using an adaptation of signal detection theory. Normal human subjects were divided into two general groups, one in which the thermal stimuli were applied during electrical stimulation of forearm nerves and a sham stimulation group in which the electrical stimulation was not applied even though the subjects were told it was. This paradigm permitted both an analysis of as well as a control for the subjective factors related to suggestion which may affect the perception of non-noxious and noxious thermal stimuli. All results were tested statistically to a significance of $p=.05$ using an analysis of variance. In addition, the experiment was repeated using two different paradigms of stimulation to ensure that the results were not likely to be paradigm specific. In previous investigations utilizing SDT in the study of experimental pain, it has often been assumed that changes in criterion without concomitant changes in discriminability reflect only changes in response bias rather than actual changes in perception. The present study indicates that in a paradigm in which the experimentor does not have explicit control of the perceptual variables, this assumption is not generally valid. Specifically, we found that a change in discriminability occurred during sham electrical stimulation, thus demonstrating that this factor could be affected by suggestion. Secondly, during actual electrical stimulation, changes in criterion without accompanying changes in discriminability were observed which were interpreted as reflecting an actual analgesia rather than changes merely in response bias, namely a change in the subject's verbal description of what was actually perceived.

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1560 BODY REPRESENTATION IN S-I CORTEX OF CAT: RELATION TO CYTOARCHITECTURE. T.M. McKenna*, D. A. Dreyer & B. L. Whitsel. Dept. of Physiol., Sch. Med. and Dent. Res. Cent., University of North Carolina, Chapel Hill, North Carolina 27514.

While the body representation in somatosensory area I (S-I) of the monkey cerebral cortex has been described in a way that permits analysis of the transformations between the peripheral sensory receptor sheet and its cortical representation, existing accounts of the cat S-I body representation lack the resolution required to perform such an analysis. As a consequence, the rules governing the mapping of the body onto the S-I cortex in these two species cannot be directly compared. With this objective in mind, we performed extracellular recordings from single neurons in the S-I cortex of unanesthetized cats. The receptive field (RF) and submodality of each neuron was determined using light punctate mechanical stimulation of the skin, hair movement, limb movement or pressure applied to deep tissues. Neurons were sampled in cytoarchitectural areas 2, 1, 3b, 3a and 4; all penetrations were histologically verified. A map of the connectivity and submodality distribution of the cat S-I cortex was obtained by plotting the data from each penetration in relation to cortical cytoarchitecture. This relationship is depicted in two ways: (1) with the gyri as they appear normally and (2) with the sulci "opened" and the cortex unfolded onto a plane. It is shown that in many respects, the connectivity and submodality distribution of the S-I map in both cat and monkey are similar. Although it frequently was observed that penetrations advanced across cytoarchitectonic boundaries did not exhibit a transition in submodality, neurons responding selectively to deep stimuli were found in greatest numbers in areas 3a and 2 and cutaneous neurons occurred in greatest numbers in areas 3b and 1. In all penetrations that crossed cell columns, a continuous sequence of RFs was observed. This not only occurred when a penetration crossed cytoarchitectural boundaries but also when it remained within a single cytoarchitectural field. The extensive cortical zones devoted to the representation of cutaneous forepaw and hindpaw mechanoreceptors each extended continuously from area 2 into area 4. Discrete RFs on the wrist or ankle were represented at multiple points around the forepaw and hindpaw regions and it appears that, as in the monkey, the distal fore- and hindlimb representations are continuously bounded by cell columns receiving input from the wrist and ankle, respectively. Observations obtained in this study lead us to conclude that, despite the many obvious differences between monkey and cat, the rules which govern those aspects of somatosensory cortical organization revealed by RF and submodality mapping appear to be identical in the two species. Supported, in part, by NIH grants NS10865, DE02668 and RR05333.

1561 SENSITIZATION OF A-DELTA NOCICEPTIVE AFFERENTS TO NOXIOUS RADIANT HEAT DELIVERED TO THE MONKEY HAND. Richard A. Meyer* James N. Campbell, and Robert H. LaMotte. Dept. Physiol., Sch. Med., The Johns Hopkins Univ., Baltimore, Md. 21205.

A group of A-delta nociceptive afferents (ADN) that innervated the hairy or glabrous skin of the monkey hand developed enhanced responsivity (sensitization) to noxious radiant heat delivered to their receptive fields. Thirty-seven ADN's (28 glabrous and 9 hairy units) with a median conduction velocity of 32 m/s, a mean receptive field area of 42.8 mm^2, and a mean mechanical threshold to nylon monofilaments of 3.89 bars were studied by the method of single unit recording. A CO_2 infrared laser under radiometer feedback control provided a step increase in skin temperature to a constant level ($\pm$ 0.1°C) over a 7.5mm diameter spot with rise rates greater than 30°/sec. Approximately half of these high threshold mechanosensitive afferents did not respond to the first presentation of 53°C, applied for 3 sec. However, with each presentation of this stimulus the number of impulses evoked progressively increased from 0 to 5 on the first trial to a mean plateau level of 58 impulses during subsequent trials. There was also a progressive decrease in response latency and the development of an afterdischarge which typically lasted several seconds after termination of the stimulus. Spontaneous activity between stimulus presentations developed in about half the units studied. Heat thresholds were tested 5 to 30 minutes after sensitization and were found to be significantly lowered - occasionally as low as 38°C. Also, the rate of discharge evoked by heat stimuli increased monotonically with increasing stimulus temperature. The dynamic response properties of these units were compared with those of 58 polymodal nociceptive C fibers (CPN) innervating the monkey hand. These CPN's had a mean conduction velocity of 0.8 m/s, a mean receptive field area of 18.9 mm^2, and a mean mechanical threshold of 5.95 bars. In contrast to the ADN's, the CPN's typically showed a decline in response to successive thermal stimuli, an increase in response latency, and an increase in heat threshold. Of 17 CPN units studied with repeated 53°C stimuli, only 3 developed signs of sensitization. These results suggest that the ADN's contribute to the increased magnitude of pain evoked by heat stimulation following a first degree burn.

1562 ACTIONS OF SOMATOSTATIN AND METHIONINE-ENKEPHALIN ON CAT DORSAL HORN NEURONES ACTIVATED BY NOXIOUS STIMULI. Vjekoslav Miletic*, Mark S. Kovacs* and Mirjana Randic. Iowa State University of Science and Technology, Ames, Ia. 50011.

Using the indirect immunofluorescence technique Hökfelt et al. (Neuroscience, 1:131, 1976) have demonstrated the occurrence of somatostatin-positive fibres in the superficial laminae of the dorsal horn, with the highest concentration observed in the lamina II of the rat spinal cord. The physiological role of somatostatin-positive fibres is at present unclear. Since it is known that this region contains neurones principally excited by an input in nociceptor afferent fibres, it was of interest to study the central effects of somatostatin by applying it microelectrophoretically to dorsal horn nocioceptive and other types of neurones at the level of Rexed's laminae I-VII. In addition, we have tested sensitivity of nocioceptive neurones in the laminae I-III to methionine-enkephalin. It is known that this area contains both, opiate receptors and enkephalins.

We have found that: 1) Somatostatin causes a depression of almost all tested dorsal horn neurones in laminae I-III, activated either by noxious mechanical and/or thermal stimulation or by a volley in Aδ or C fibres. In addition, the wide dynamic-range cells located in lamina V, activated by both non-noxious and noxious cutaneous stimuli, were moderately depressed by somatostatin. Occasionally biphasic responses were seen in lamina V-type cells. 2) Somatostatin depressed responses of nocioceptive neurons to adequate stimulation. 3) In contrast, the majority of units activated by low-threshold mechanical and electrical stimuli located in laminae I-V were unaffected by somatostatin. 4) Infrequent excitatory effects of somatostatin were observed in lamina VII. The characteristics of the depressant response to somatostatin were as follows: relatively rapid onset and recovery, depression of rate of firing was frequently accompanied with the increase in spike amplitude, graded decrease in excitability with increased current and absence of similar response during application of the control positive current.

The predominant effect of methionine-enkephalin in nocioceptive units was depression of spontaneous and evoked neuronal firing rate. A few units activated by low-threshold mechanical stimuli seem to be insensitive to methionine-enkephalin.

Our results demonstrating the depressant effect of somatostatin upon the majority of tested spinal nocioceptive neurones seem to suggest that somatostatin-containing neurones may exert an attenuating influence upon the incoming pain information. In addition, we have shown that methionine-enkephalin has a potent depressant action on the excitability of nocioceptive neurones located at the level of Rexed's laminae I-II. (Supported by PHS Grant NS12972-01 and Iowa State University Research Foundation).

1563 IDENTIFICATION OF CORNEAL AFFERENT CELLS IN THE TRIGEMINAL GANGLION OF THE CAT USING HORSERADISH PEROXIDASE. Charles Morgan, Irving Nadelhaft, William C. de Groat. Depts. of Neurosurg. and Pharmacol., Univ. of Pittsburgh School of Medicine and Veterans Administration Hospital, Pittsburgh, Pa., 15261.

Afferent fibers from the cornea travel through the trigeminal nerve into the brain stem. It has been generally assumed that the cell bodies of these fibers are located in the trigeminal ganglion; however, this has never been definitely established.

In the present study corneal afferent cells have been positively identified in the ophthalmic region of the trigeminal ganglia of seven cats. The basic procedure involved scoring the cornea of one eye with a knife, applying horseradish peroxidase (HRP) to both eyes, and then covering both eyes with contact lenses. The cats were anesthetized with dial urethane throughout the whole procedure including the 30-36 hours allowed for transport of the HRP to the ganglion cells. Frozen sections, 42 u thick, were processed in diaminobenzidene or benzidene after being mounted on glass slides. Cells were identified under dark field illumination.

In the control eyes the HRP had contact with the intact cornea, sclera, conjunctiva, and eyelids, yet no cells were labelled in the ipsilateral ganglia. On the basis of this it seems reasonable to conclude that HRP was only taken up by the nerve endings of the damaged cornea and not by the intact cornea or surrounding mucous membranes. The number of cells labelled was roughly proportional to the amount of area damaged. A 9 mm diameter circular area of damage commonly led to labelling of 75 to 100 cells, whereas scoring the entire cornea (approx. 5 times greater area) resulted in 691 labelled cells in one cat. Cells were distributed randomly only within the medial portion of the ganglion in an area 3 mm long, 1 mm wide and 1.9 mm thick extending from dorsal to ventral surfaces. Scoring medial or lateral portions of cornea did not result in any obvious shift in the random distribution of labelled cells within the ganglion and suggests there is no topographical organization to this system at this level. The labelled cells were typical monopolar sensory ganglion cells ranging from 20 to 50 microns in diameter.

Within the limits of this experiment we conclude that corneal afferents are carried in the trigeminal nerve and have their cell bodies randomly distributed throughout the medial portion (ophthalmic area) of the trigeminal ganglion in a non-topographic manner. We find that the nerve endings in intact corneal tissue do not take up HRP but must be damaged for this to occur. The number of cells labelled is proportional to the damage.

1564 THE EFFECT OF CHRONIC TRACTOTOMY UPON THE RESPONSE PATTERNS OF SENSORY NEURONS IN ROSTRAL TRIGEMINAL NUCLEI. Samuel G. Nord and Ronald F. Young. Departments of Neurology and Neurosurgery Upstate Medical Center, Syracuse, NY 13210.

The results of previous electrophysiologic investigations of tooth pulp projections to the trigeminal sensory complex have led to the proposal that the firing patterns of cells in rostral nuclei of the complex are influenced by activity evoked in cells of the nucleus caudalis. Specifically, it has been proposed that the responses of the rostral cells to pulpal stimuli include late components which originate in the caudalis neurons. These late components have been presumed to relate to the identification of a stimulus as noxious. The present investigation examines the potential contribution of caudalis neurons to the firing patterns of the rostral trigeminal cells in experiments with chronically tractotomized animals. Adult cats were subjected to unilateral trigeminal tractotomy at the level of the obex. Four to six weeks later each animal was lightly anesthetized (Pentobarbitol) and paralyzed (Gallamine) and was studied in acute experiments. Unit activity was recorded from neurons in the trigeminal nuclei oralis and principalis on both the tractotomized and unoperated (control) sides of each preparation. Recording sites and completeness of tractotomies were verified histologically. Response patterns exhibited by cells on each side of the brainstem varied considerably. For example, single, maximally effective, pulpal stimuli evoked between one and twenty spikes in neurons located on each side. These spikes were arranged in brief bursts, in prolonged continuous trains or in multiple component discharges. Mechanical stimulation was also effective in activating many of the neurons. Again, brief, prolonged and multiple component responses were recorded. Differences in response patterns on the two sides were not detected in either the exclusively pulpal or the polymodal neurons. Thus, the results of our experiments indicate that late discharge components, whether in response to pulpal or to mechanical stimuli, may not originate in nucleus caudalis. Furthermore, our data lend no support to the hypothesis that prolonged or multiple component neuronal activity in rostral trigeminal nuclei is exclusively related to noxious pulpal stimulation.

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1565 ANALGESIC EFFECTIVENESS OF BRAIN STIMULATION IN PRIMATES ON ESCAPE BEHAVIOR TO TOOTH PULP STIMULATION. T. D. Oleson, D. B. Kirkpatrick*, and S. J. Goodman. Dept. of Surg./Neurosurg., Sch. Med., UCLA - Harbor General Hospital Campus, Torrance, CA 90509

Nine adult Rhesus monkeys (Macaca mulatta) were implanted with bipolar stimulating electrodes in the upper canines and in various regions of the brain. In four monkeys, silver disc stimulating electrodes were placed under the infraorbital neocortex. The remaining five monkeys were implanted with four electrode-track arrays aimed for midline sites in the diencephalon, mesencephalon, cerebellum, and medulla oblongata. All monkeys were trained to press a lever to terminate 5 Hz, 0.5 msec, square wave, shock pulses delivered to the tooth; current intensities were varied between 0.25 ma and 5.0 ma. These animals were also given a shock titration schedule whereby the current intensity was increased in 0.25 ma steps every 5 sec unless the monkey pressed the lever to reduce the current one step for each bar press. After current levels eliciting reliable escape and titration behaviors were established, 20 Hz, 0.5 msec, biphasic, square waves were delivered to the various brain sites for 60 sec. Electrical stimulation of the diencephalic nucleus anterior medialis, the lateral hypothalamus, the preoptic area, and the septal nucleus all elicited large and prolonged increasesin both escape and titration behavior to tooth pulp stimulation. At current intensities (1.0 ma to 3.0 ma) which elicited little to no side effects, shock thresholds were increased by over 300% and often did not return to control levels until 3 hours following the termination of brain stimulation. Only a moderate and brief elevation of shock threshold was observed during orbital cortex stimulation, and this effect was often accompanied by nonaversive but behaviorally distracting lateral eye gazes. Unlike previous reports in rats and cats, the midbrain central gray, the dorsal raphe nucleus, and the nucleus raphe magnus were not optimal sites for obtaining stimulation - produced analgesia in primates. When elevations in shock threshold occurred following electrical activation of these brain areas, they were always accompanied by aversive behaviors. Stimulation of the cerebellum resulted in similar negative findings. Naloxone (2 mg/kg) did not block the analgesic effectiveness of any brain site. This data thus points to the importance of the rostral, midline diencephalon for reducing escape responses to tooth pulp stimulation. Electrical activation of these same diencephalic areas also inhibited behavioral responses to foot shock and tail pinch, attesting to the generality of the antinociceptive effect of brain stimulation.

1566 RESPONSES OF BULBAR RETICULAR NEURONES TO REPEATED CUTANEOUS STIMULATION. J.A. Pearson* and W.M. Smith* (SPON: J.P.J. Pinel). Dept. of Physiology, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

In previous experiments designed to elucidate the neuronal basis of flexor reflex habituation, several types of spinal interneurones were encountered whose activity changed as a consequence of stimulus repetition. The type which was of particular interest was spontaneously active and was inhibited by cutaneous stimulation. The period of inhibition progressively lengthened with each successive trial. This build-up of inhibition could not be demonstrated in rats whose spinal cords had been transected at the mid-thoracic level. Furthermore, Haber and Wagman (1974) have shown that repeated stimulation of the nucleus reticularis gigantocellularis (NGC) also caused a progressive inhibition of spinal interneurones. It is suggested therefore, that NGC might be necessary for the demonstration of inhibitory build-up of spinal interneurones to repeated cutaneous stimulation.

In the present experiments the responses of neurones in the medullary and pontine reticular formation were studied. Rats were anaesthetized with urethane (1.5 g/kg). Unitary activity of reticular neurones was recorded using glass microelectrodes containing pontamine sky blue (4% in 1M NaCl). Recording sites were marked by ejection of the dye from the recording electrode. Peripheral cutaneous stimuli were delivered through a pair of 30 gauge needles inserted into the skin of a hind paw.

The activity of 35 neurones, which were situated either in the NGC or the nucleus reticularis pontis caudalis (NPC) and activated by cutaneous stimulation, was studied in detail. The threshold for activation of these cells was high (10-15V., 0.5 msec) and the response occurred after a long latency (20-50 msec). Weak natural stimuli (hair movement, touch) were ineffective and strong pinch or pin prick were required. On repeated stimulation, the responses of 14/35 cells remained constant in latency and duration. In the remaining 21 cells, the duration of the evoked discharge progressively increased with repeated trials and often persisted for several seconds after the stimulation ceased. The rate of build-up of activity was directly related to both the intensity and the frequency of stimulation. Eight of these 21 cells could be antidromically activated by stimulation (via concentric bipolar electrodes) of the lumbar cord.

In summary, repeated cutaneous stimulation causes a progressive increase in the activity of reticular neurones whose axons project to the lumbar cord. It is tentatively suggested that this response pattern may be partially responsible for the development of inhibition of spinal interneurones.
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1567 PROCESSING OF CUTANEOUS TEMPERATURE INFORMATION IN CAT MEDULLA, D.A. Poulos, E.L. Auen*, J.T. Molt and H. Hirata*, Div. of Neurosurgery and Dept. of Physiology, Albany Medical College, Albany, N.Y. 12208

We have studied further the response properties of one population of thermoreceptive afferents identified in the spinal trigeminal nucleus of urethane anesthetized cats whose peripheral receptive fields appear to be limited to the glabrous surfaces of the nose and lips (Molt, J.T. and Poulos, D.A. (1976) Neurosci. Abstr. Vol. II p 945). The units were considered to be specific cold afferents; however, their response characteristics differed somewhat from the usual description of trigeminal cold receptors. Unlike the "typical" cold receptors we found that give dynamic rate increases to rapid cooling steps of 2-20° below a 35°C standard temperature, the units studied did not increase their activity above the preceding 35°C firing level and indeed more often showed a transient (2-3 sec) rate decrease to rapid cooling. Like "typical" cold receptors, the units did respond to rapid warming steps of 2-10° above the standard 35°C with transient rate decreases and surprisingly, showed dynamic rate increases when returned (cooled) to 35°C from all warmer stimulus temperatures. Due to the latter observation we re-examined the behavior of this unit type to rapid cooling below a warmer standard temperature of 43°C. When rapid cooling steps of 2-28° were applied below the warmer 43°C standard temperature the units behaved like typical cold receptors in that dynamic rate increases always occurred. The dependency of the type of dynamic response to cooling seen in these units on starting temperature cannot be attributed to differences in primary afferent input. All primary afferent cold fibers thus far described invariably show rate increases upon rapid cooling. Nor can the differences we observed be attributed to an overall shift in the range of thermal sensitivity of the cells since their static temperature response profiles tested over a wide range of temperatures held constant (45-13°C) are indistinguishable from those of typical thermoreceptors. We conclude that a form of neural processing occurs within the marginal zone of the spinal trigeminal nucleus where these cells are found intermingled with the population of typical thermoreceptors. Since their peripheral receptive fields appear to be limited to glabrous skin, the cells we have described may play some special and thus far unrecognized role in the central processing of thermal information.
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1568 NEURAL REPRESENTATION OF TACTILE-EVOKED AFTERSENSATIONS BY SPINOTHALAMIC TRACT NEURONS. D. D. Price, R. L. Hayes, M. A. Ruda, and R. Dubner. Neurobiology and Anesthesiology Br., NIDR/NIH, Bethesda, MD 20014.

Long duration aftersensations can be evoked by brief tactile stimuli. They outlast the duration of the stimulus and arrival of primary afferent input by several seconds and often minutes. They are often aversive and can be immediately terminated by rubbing the stimulated area. The possible involvement of the spinothalamic tract in this phenomenon was investigated by analysis of tactile-evoked responses of 45 spinothalamic tract neurons and of 20 presumed interneurons of L-7 of rhesus monkeys. They were maintained on 66 percent nitrous oxide and 34 percent oxygen and their carotid arteries were bilaterally ligated. Each cell was tested for afterresponses to a variety of natural stimuli as well as electrical stimulation of receptive fields. A mechanical stimulus used to test all neurons consisted of moving a camel hair brush 5 cm in 5 sec along the major axis of receptive fields. As in previous studies, we found neurons that had input from low threshold mechanoreceptive afferents (tactile neurons), neurons that had input from low threshold afferents and nociceptive afferents (wide dynamic range neurons, WDR), and neurons that had nearly exclusive input from nociceptive afferents (nociceptive specific neurons, NS). None of 20 tactile neurons responded with afterresponses to the camel hair brush stimulus, to noxious heat, or to intense electrical stimulation. Four of these were spinothalamic neurons. In contrast, 13 of 18 WDR spinothalamic neurons responded with distinct afterresponses (20-56 sec duration) to the standard camel hair brush stimulus. These afterresponses could be abruptly terminated by firmly rubbing the stimulated area. The locations of neurons exhibiting this response were in dorsal horn laminae I, IV, and V. Only noxious stimuli evoked afterresponses in NS neurons. Thus, input over WDR neurons may be necessary for tactile-evoked aftersensations in primates.

1569 SOMATOTOPIC ORGANIZATION OF THE DORSAL HORN IN CATS WITH PARTIAL LUMBOSACRAL DEAFFERENTATIONS. Lillian M. Pubols and Michael E. Goldberger. Dept. Anat., Med. Coll. of Pa., Phila., Pa. 19129.

The receptive fields of dorsal horn neurons at the L6 spinal cord segment in cats are organized such that the proximal hindlimb is represented laterally; the distal hindlimb, medially (Brown & Fuchs, J. Neurophysiol. 39:1, 1975). The L6 dermatome does not extend as far proximally as the receptive fields of the most laterally placed dorsal horn neurons (Kuhn, J. Neurophysiol. 16:169, 1953). Therefore, these neurons are thought to be innervated by roots other than L6. In view of recent evidence that the receptive field characteristics of dorsal horn neurons may be altered following partial deafferentation (Basbaum & Wall, Brain Res. 116:181, 1976), a study of the somatotopic organization of the L6 dorsal horn of L6-spared, lumbosacral deafferented cats was undertaken.

Unilateral lumbosacral dorsal rhizotomies were performed extradurally. Microelectrodes were used to map the tactile input to dorsal horn neurons at L6 in methoxyflurane anesthetized cats. Four animals in which all roots caudal to T12 were sectioned, sparing L6, were studied, one each at survival times of 12 hours, 2, 9 and 30 days. The results from these animals were compared with maps from two animals having both the L6 and L7 roots intact at 2 day survivals, and with published data from normal animals.

In animals having a single root spared receptive fields were exclusively on the lower leg and foot. The lateral foot, including digit 5, was not represented. All but one of the responsive loci were located in the medial 2/3 of the dorsal horn. In animals with two roots spared there were significantly more responsive loci per active electrode penetration than in the one root animals. This difference was seen throughout the mediolateral extent of the dorsal horn. The somatotopic map of two root animals was similar to that of one root animals, but extended to the lateral border of the dorsal horn. In two root animals some neurons with large, multiple receptive fields, which always included an area adjacent to the dorsal midline, were seen. These neurons tended to be located close to the border of the lateral white column.

Thus, in both types of deafferented preparations the somatotopic map of the L6 dorsal horn is consistent with that of intact animals. The L7 root supplies the lateral one-third of the dorsal horn, as well as the medial 2/3 also innervated by L6.

1570 RESPONSES TO SOMATIC STIMULATION IN N. PARAFASCICULARIS (Pf) AND IN THE POSTERIOR GROUP OF NUCLEI (PO) IN THE UNANESTHETIZED CAT. W. W. Pugh* and I. H. Wagman. Department of Animal Physiology, University of California, Davis, California 95616.

Single unit electrical activity was recorded from immobilized cats by means of chronically implanted chambers. Attachment of the head to the stereotaxic frame by an adapter permitted painless fixation while maintaining stability. 213 units (75 in PO and 70 in Pf were confirmed histologically) were analyzed. Stimuli used were electric to skin as well as adequate ranging from hair movement to strong pinch of skin and deep tissue. Responses of the two nuclei differed as follows: (1) *Receptive field (RF) size and symmetry.* 39% of PO cells had bilateral receptive fields covering most of the body surface. The remaining RFs were contralateral ranging from one limb (23%) to less than 2 cm^2 (38%). 90% of Pf units had bilateral RFs which involved either most of the body surface or both extremities. A few cells (10%) had contralateral receptive fields which were also quite large. No somatotopical organization was noted for either nucleus. (2) *Latency of response.* Following threshold electric skin stimulation, units in PO responded with a bimodal distribution of latencies. 63% of cells responded with an average latency of 20 ± 4.9 msec; the remainder had an average latency of 36 ± 3.2 msec. Similarly 62% of Pf units had an average latency of 20 ± 3.6 msec. but the others had latencies varying from 40 to 800 msec. (3) *Modality and dynamic range.* The PO group had 20% audio input of which nearly half also responded to somatic stimuli. No cells in Pf were excited by auditory stimulation. Responses in both PO and Pf were obtained by adequate stimulation: hair displacement, touch, brisk tap, skin and deep tissue mechanical stimuli, joint rotation. However, 60% of PO cells responded to low threshold somatic stimuli while 10% of Pf units responded similarly. Brisk tap affected 5% of PO and 62% of Pf cells. Intense mechanical stimulation including extremes of joint movement excited 15% of PO and 37% of Pf cells. Two-thirds of the former and one-half of the latter had wide dynamic ranges, i.e., they responded to low intensity stimuli or tap as well. (4) *Inhibition.* Under the conditions of the experiment only a few PO cells were inhibited, usually by light mechanical displacement of skin or hair movement in or near the excitatory RF. In contrast, a small number of Pf cells also showed inhibition, but only by intense stimulation such as extreme flexion/extension of joints or strong pinch to skin or deep tissue. In summary, in the unanesthetized cat Pf is more likely to be excited by poorly localized tap, articular or intense deep tissue and skin stimulation while the PO group responds more often to more localized light cutaneous input. (Supported in part by NIH grants RR00169 and AM16716).

1571 THALAMIC NEURONS PROJECTING TO THE SECOND SOMESTHETIC *AREA (SII)* OF THE CORTEX OF THE VIRGINIA OPOSSUM. Martine J. Robards and Ford F. Ebner. Neuroscience Sect., Div. Bio. & Med., Brown Univ., Providence, R. I., 02912.

Large and small iontophoretic injections of horseradish peroxidase (HRP) were placed in electrophysiologically identified regions of the SII cortex of halothane-anaesthetized opossums. After varying survival intervals (mode: 48 hours), the animals were sacrificed, and sections through thalamus were reacted with diaminobenzidine and peroxide in a phosphate buffer.

Receptive fields of units in SII were found to be large and predominantly bilateral (although not necessarily symmetric)in both forelimb and hindlimb representation areas. When low impedance micropipettes (500 KΩ) were used, click-evoked potentials could be recorded in SII, but with higher impedance micropipettes (2 MΩ), which readily recorded somesthetic unit activity, no units were found in SII which could be driven with a click stimulus.

HRP injections in electrophysiologically localized forelimb regions filled a large number of neurons throughout the middle third of the ventrobasal complex (VB), the classical forelimb area. In addition, clusters of cells in the magnocellular medial geniculate (MG_{mc}), lateral and medial parts of the posterior group, ventromedial nucleus, and the central intralaminar nucleus were filled.

HRP injections in electrophysiologically localized hindlimb regions filled a large number of neurons in the lateral third of VB (classical hindlimb area), as well as cells in the other nuclei listed above. However, while the distribution of filled cells in VB was different after fore- and hindlimb SII injections, no similar distinction between the two types of injections could be made in the other thalamic nuclei.

Cases which showed filled cells in VB always filled cells in the other nuclei, and vice versa. Hindlimb injections in SII tended to produce a relatively greater number of filled cells in the non-VB thalamic nuclei, especially in MG_{mc}. Since the hindlimb representation abuts on the auditory cortex, and since we saw an occasional filled cell in the ventral (or principal) nucleus of the medial geniculate in hindlimb SII injection cases, we cannot be certain that the injection did not spread into the adjacent fringe of auditory cortex. Alternatively, hindlimb SII may receive a relatively greater input from non-VB nuclei than does forelimb SII.

No evidence, either anatomical or electrophysiological, was found to support a division of SII of opossum into the "place-modality-specific" and "place-modality-nonspecific" subdivisions as reported for cat. (Supported by USPHS #NS13031.)

1572 SYMPATHETIC INFLUENCE ON RESPONSES OF FROG CUTANEOUS RECEPTORS. William J. Roberts and Anne L. Calof*, Neurological Sciences Institute, Good Samaritan Hospital, Portland, Oregon 97209.

Localized sympathetic dysfunction and pain are often coincident in patients; however, there is no accepted explanation of the function of the sympathetic nervous system in chronic or acute pain except in certain syndromes. These experiments have been designed to investigate the possible modulation of nociception by sympathetic actions on receptors. The initial work has focused upon low-threshold mechanoreceptors, which are thought to be involved in nociception through their inhibitory actions on unmyelinated fibers in the spinal cord.

Unitary activity in afferent fibers was recorded from filaments of the cutaneous branch of the peroneal nerve in Rana pipiens. Afferent units were first characterized as to their preferred (adequate) stimulus. Calibrated mechanical stimuli, consisting of step changes in force, were then applied to the skin with a solenoid device. Spike threshold and adaptation rate were thus measured.

Effects of sympathetic stimulation were consistent for receptors of a given type, adaptation rate and mechanical threshold. Fast adapting compression receptors with very low thresholds (< 70 mg force from a 3 mm dia. probe) were inhibited, showing threshold increases on the order of 25%. This inhibition was maintained during low frequency (5/sec) sympathetic stimulation. Fast-adapting compression receptors having higher thresholds (80-300 mg) were briefly facilitated, but this facilitation did not persist with maintained sympathetic stimulation. Slowly adapting compression receptors were also briefly facilitated by sympathetic stimulation. "Stroke" receptors showed a prolonged facilitation which differed from the facilitation of compression receptors in that it did persist with maintained sympathetic stimulation. No consistent sympathetic influence on myelinated prick receptors has been observed. The effects described above are the predominant effects observed; some units showed a brief facilitation-inhibition sequence. C-fiber nociceptors have not yet been tested.

These results indicate that sympathetic modulation of mechanoreceptor sensitivity is consistently observed in frog skin with an intact blood supply. There are transient effects, lasting several seconds, and maintained effects; both are consistent for each class of receptor as defined above. Prediction of the role of the sympathetic system in nociception requires, in addition to these data, knowledge of which classes of low threshold receptors influence nociception centrally, and information about sympathetic actions on non-myelinated afferents.

1573 BRAINSTEM UNIT RESPONSES TO TACTILE, NOCICEPTIVE AND GENITAL STIMULI IN FEMALE SQUIRREL MONKEYS. James D. Rose. Depts. Psychiat. and Anat., Sch. Med., Emory Univ., Atlanta, GA 30322.

There is much current interest in mechanisms of somatic sensation and pain in primates, but research has focussed on the spinal cord, thalamus and neocortex. Contemporary views of the sensory properties of reticular neurons are based largely on data from cats and rodents. The present work provides information on the sensory responses of brainstem reticular neurons in the monkey. In 13 female squirrel monkeys anesthetized with a chloralose-urethane mixture and paralyzed with gallamine triethiodide, 317 brainstem single units were tested for responsiveness to the following stimuli: tactile stimulation of the face, limbs, tail, trunk and external genitalia; vaginal and rectal probing; and nociceptive stimuli including foot pinch, needle pricks and localized heat. Tactile, genital and nociceptive stimuli elicited responses in 68, 69 and 64% of the units, respectively. Responsive neurons were located in the reticular formation, extending from the caudal medulla to the posterior thalamus and in non-reticular (e.g. tectum) regions as well. The unit responses consisted primarily of simple accelerative, and less often decelerative, changes in firing. Most of the cells responded to more than one form of stimulation. Units responding to tactile stimuli had extensive, usually bilateral peripheral receptive fields. Responses to vaginal stimuli were usually simple accelerations of firing which, in some instances, were enhanced by probe contact with the cervix. Responses to a foot pinch were most pronounced at pinch intensities which were well above the threshold for flexion responses in lightly-anesthetized unparalyzed monkeys. These nociceptive responses displayed little or no linear correlation with pinch force. In contrast to the good responsiveness of brainstem units to pinch stimuli, they were quite unaffected by needle pricks and thermal stimulation. In 6 of the monkeys, the effect of estradiol administration on the responsiveness of midbrain and pontine units was examined. The proportion of cells responding to vaginal probing was increased by estradiol, and similar to results reported for the cat by Rose (Brain Res. 97: 79, 1975; Exp. Neurol. 49: 639, 1975) the number of units responsive to tactile stimuli was increased as well, but the hormone had no effect on unit responsiveness to nociceptive stimuli. Results in the monkey differed from those in the cat in that genital tract stimulation didn't elicit a complex array of unit response patterns, no units responded exclusively to genital stimulation and estrogenization did not enlarge tactile receptive fields of monkey brainstem units. Supported by NIH Grant NS12260.

1574 DIRECT COMPARISON OF HUMAN AND MONKEY TEMPERATURE SENSITIVITY. Andrew J. Rózsa, Helen H. Molinari, and Dan R. Kenshalo. Department of Psychology, Florida State University, Tallahassee, Fla. 32306.

The thermal sensitivities of three human subjects and two rhesus monkeys have been examined using a signal detection "yes-no" procedure. The experimental paradigm used for the two species was identical except for a difference in the actual reward for correct responses. The stimuli were presented to 7 cm^2 areas on the subjects' palms from a number of adapting temperatures between 28° and 40°C.

At each adapting temperature the warm and cool stimulus intensities required to produce a constant level of sensitivity were calculated. The stimulus intensity required to produce a constant warm sensation level decreased when the adapting temperature was increased. The stimulus intensity required to produce a constant cool sensation level decreased when the adapting temperature was decreased. Since identical procedures were used for both species, direct comparisons of monkey and human thermal sensitivity can be made. Equivalent stimulus intensities produced identical warm and cool sensation levels in both species. The changes in thermal sensitivity with adapting temperature were also remarkably similar. Thus, the differences, if any, between human and monkey thermal sensitivities appear to be minimal.

These data indicate that the thermosensory neural mechanisms of the rhesus monkey can, in fact, be used as an adequate model for the human temperature sensing system.

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1575 EM AUTORADIOGRAPHIC LOCALIZATION OF INDOLEAMINERGIC AXONAL ENDINGS IN TRIGEMINAL NUCLEUS CAUDALIS. M.A. Ruda and S. Gobel. Neurobiology & Anesthesiology Br., NIDR, NIH, Bethesda, Md. 20014

Anatomical and physiological studies have demonstrated a descending inhibitory pathway to nociceptive neurons in nucleus caudalis (CAUD) and the dorsal horn of the spinal cord. This pathway is thought to be serotonergic. The purpose of these experiments was to locate and characterize serotonergic axonal endings in CAUD which might inhibit the firing of neurons in trigeminal pain pathways.

Tritiated serotonin ([^{3}H]5HT), at a concentration of 10^{-5}-10^{-6}M, was topically applied for a period of 1 hr. onto CAUD of adult cats pretreated with a monoamine oxidase inhibitor to block the catabolism of serotonin and anesthetized with sodium pentobarbital. Our approach relies on the ability of neurons which normally utilize serotonin as a neurotransmitter to take up [^{3}H]5HT at their axonal endings. Following a survival time of 30 min-1 hr., the cats were perfused with 1% glutaraldehyde and 1% paraformaldehyde in a phosphate buffer. CAUD was then processed for light and EM autoradiography. Light microscopic analysis of 1μm sections demonstrated a specific laminar distribution of [^{3}H]5HT. Layer I was heavily labelled while layer II was moderately labelled. Grain density in layer III and the magnocellular layer was not above background level.

In EM autoradiographs, two major categories of morphologically distinguishable axonal endings were found to contain [^{3}H]5HT: dome-shaped endings and scalloped endings. Most of the dome-shaped endings were located in layer I. These endings formed single synapses, predominantly on small caliber dendritic shafts. The most heavily labelled group of dome-shape endings contained small, highly flattened, agranular vesicles and a few dense core vesicles. A second group contained small round or oval agranular vesicles and a few dense core vesicles. In contrast, the scalloped endings were found predominantly in layer II. These endings formed multiple synaptic contacts with several small caliber dendritic shafts and spines. They contained round or oval agranular vesicles and an occasional dense core vesicle.

Since our Golgi and HRP studies have shown that most of the dendrites in layer I are derived from layer I projection neurons, these experiments indicate a direct axodendritic serotonergic input to layer I projection neurons. In addition, serotonergic inputs to layer II synapse on dendrites of interneurons which modulate the transfer of input from primary axonal endings in layers II and III to the projection neurons in layer I. These inputs to layers I and II may represent descending pathways for the modulation of layer I nociceptive neurons by one or more serotonergic cell groups.

1576 CELLULAR CONDENSATIONS IN SENSORIMOTOR NEOCORTEX OF THE OPOSSUM (Didelphis virginiana). S.T. Sakai* and W.L. Weller (SPON: L.I. O'Kelly) Dept. of Psychology and Neuroscience Program, Michigan State University, East Lansing, MI. 48824 and Dept. of Anatomy, University of Tasmania, Hobart, Tasmania 7001.

Discrete cytoarchitectonic units termed barrels have been reported in SmI cortex of the mouse (Woolsey and Van der Loos, Brain Research, 17: 205, 1970). The brains of several mammals have since been examined in order to determine whether similar cellular condensations occur in the SmI face area (Woolsey, Welker and Schwartz, J. Comp. Neurol., 164: 79, 1975). The present study investigated tangential sections of the opossum sensorimotor cortex. Because cellular condensations may be more readily visualized in young animals, brains were obtained from 30, 40, 58, 70 day and adult opossums. The tissue was fixed in aldehydes and embedded in celloidin. Fifty μm tangential sections were stained in thionin. Cellular condensations were observed in layer IV in the 30, 40, 58, 70 day and adult opossum sensorimotor cortex. These condensations were oriented perpendicular to the pial surface and appeared as cell-dense circular or ellipsoidal rings containing fewer cells in the hollow. A septum of relatively fewer cells separated adjacent cell rings. The diameter of the cell ring ranged from 85 to 150 μm. The number of cellular aggregates ranged from 128 in the 30 day old opossum to 616 in the adult opossum. Three major groups of cellular aggregates appeared to be present in the rostromedial, rostrolateral and caudal areas of the sensorimotor cortex. Furthermore, these major groups appear to be connected by bridges of sparse cellular aggregates.

Because previous work indicated that the barrels of the posteriomedial barrel subfield in the rat functionally correspond to the mystacial vibrissae representation (Welker, Brain Research, 26: 259, 1971), it is interesting to note that the spatial distribution of the cellular condensations does not appear to be confined to the facial representation as outlined electrophysiologically by Pubols, Pubols, DiPette and Sheely (J. Comp. Neurol., 165: 229, 1976). Furthermore, the number of cellular condensations (128-616) far exceeds the number of vibrissae present in the opossum (46-56) as reported by Lyne (Proc. Zoo. Soc., 133: 79, 1969). Possibly some groups of cellular condensations correspond to other peripheral structures. However, the functional correlate is yet to be investigated.

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1577 SPINAL TRIGEMINAL TRACT IN DOGS: AN ELECTROPHYSIOLOGICAL STUDY. Richard J. Schneider, Fred Nelson*, Henry Wiener* and Abdul L. Itani*. Dept. Surg., Div. Neurosurg. U. Md. Med. Sch., Balt., Md. 21201.

Insulated tungsten microelectrodes with electrolytically sharpened tips (1-20μ) were used to record evoked potentials from single cells and nerve fibers in the descending trigeminal tract and nucleus. Physiological stimuli (light touch, pinprick) were employed to initiate these potentials, while their conduction velocity was measured with electrical stimulation. Histology of several brainstems in this series was done.

The initial question we were asking was whether the representation of the head in the spinal trigeminal tract and nucleus conformed to the Dejerine onion-skin pattern. This pattern was deduced clinically from the distribution of sensory loss on the head following spinal trigeminal tractotomy for intractable pain in human patients. It centers on the nose and lips and expands in circles of increasing radius in lamellar fashion to include the rest of the head. This differs from the pattern of representation described for the three divisions of the trigeminal nerve: mandibular, maxillary and opthalmic. In the latter, a zone of innervation corresponding to a particular rostro-caudal division should be spared by a tractotomy at different levels. In addition, physiological characteristics of the cells and fibers were sought.

In dogs, the divisional pattern of representation was seen to exist in the descending tract. A submodality segregation (hair, skin, pressure) may also exist. Latencies to electrical stimulation indicated conduction velocities in the Group II range. The majority of units conducted in this range while only a few would fall into the conduction velocity group associated with "C" fibers and noxious stimuli.

1578 CHARACTERISTICS OF SOMATIC RECEPTIVE FIELDS OF NEURONS IN POSTCRUCIATE CEREBRAL CORTEX IN AWAKE-RESTRAINED AND TWO ANESTHETIC CONDITIONS IN THE SAME CAT. J.C. Slimp and A.L. Towe. Dept. Physiol. & Biophys. (SJ-40), Univ. of Wash. Sch. of Med., Seattle, WA 98195.

Evidently the characteristics of somatic input to the cerebral cortex differ under different anesthetic conditions. To examine these characteristics and compare them with the unanesthetized condition, a cat was prepared for chronic extracellular recording. The location, size and modality of somatic receptive fields of postcruciate cerebral neurons were studied under three conditions: unanesthetized (542 neurons), chloralose anesthesia (84 neurons) and sodium pentobarbital anesthesia (142 neurons), over a seven-month period. Of 64 sites sampled in the unanesthetized condition over a 20 mm^2 area, 17 were shared in common with the two anesthetic conditions. Half of the neurons in barbiturate, one-fourth in unanesthetized and one-eighth in chloralose, though tonically active, were unresponsive to somatic stimulation. Of the responsive neurons, the percentage distributions by modalities for the 17 common sites are shown in the table below, along with that for the 64 sites (in parentheses) for the unanesthetized condition.

Modality/Condition	Pentobarbital	Unanesthetized	Chloralose
Touch	61	35 (38)	67
Hair	10	13 (13)	25
MTJ	24	27 (23)	8
Joint only	4	24 (26)	0

Although the locations of the receptive fields did not differ markedly among the three conditions, the sizes differed in several respects. Receptive fields in chloralose were larger and those in barbiturate were smaller than in the unanesthetized condition. All of those in barbiturate, nine-tenths of those in unanesthetized and three-fourths of those in chloralose were less than a tenth of the body surface in area. Bilateral receptive fields were not found in barbiturate, though they made up 3% of the unanesthetized and 21% of the chloralose samples. In the unanesthetized condition, the size of the excitatory receptive field and the degree of responsiveness of the neuron changed with the state of alertness and/or simultaneous stimulation outside the excitatory receptive field on one-seventh of the neurons. Thus, the response characteristics of individual cerebral neurons and the statistical properties of samples of cerebral neurons vary with the experimental conditions. Even in unanesthetized preparations, attention must be paid to the posture and state of alertness of the animal.

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1579 BIPHASIC ALTERATIONS OF NOCICEPTIVE THRESHOLDS INDUCED BY FOOD DEPRIVATION. Angela Spiaggia*, Richard J. Bodnar, Dennis D. Kelly, Mary Ellen McManus*, and Murray Glusman. New York State Psychiatric Institute, New York, N.Y. 10032.

An abrupt shift to a starvation diet is a severe stressor which prompts a variety of physiological reactions and behavioral adaptations, such as increased ACTH secretions, accelerated motor activity and pronounced adjunctive behaviors. The present study investigated whether food deprivation like other stressors would alter respondent nociceptive thresholds. The flinch-jump thresholds of 16 rats were tested in twice daily sessions for three baseline, three food deprivation, and two recovery days. Weight, food intake, water intake and activity were also monitored. All rats were maintained on a 14 hour on/10 off light-dark cycle. For eight rats deprivation was scheduled to begin at the end of the dark cycle, while the other eight began at the end of the light cycle. After 12 hours, on the first day of deprivation, jump thresholds were significantly higher than normal, while after 72 hours on the third day of deprivation, jump thresholds were significantly lower than baseline. These biphasic changes in jump thresholds were most marked in rats tested at the end of their dark cycle. Although baseline food intake, water intake and activity were also greater at the end of the dark cycle than at the end of the light cycle, the biphasic alterations in nociceptive thresholds during food deprivation could not be predicted by changes in these variables. These data demonstrate that food deprivation can cause orderly alterations in nociceptive thresholds which are sensitive to circadian rhythms but which are independent of such factors as water intake, activity and weight. Viewed in the context of other studies of stress-produced analgesia, it seems likely that an abrupt shift to a starvation diet may trigger a series of neurochemical events which, in the short term, produced analgesia, but which, over a longer period, render the organism hyperalgesic. (Supported by NIMH Grant #13579 and by New York State Health Research Council Grant #365.)

1580 THE REPRESENTATION OF THE BODY SURFACE IN SmII OF THE GREY SQUIRREL. M. Sur*, R. J. Nelson and J. H. Kaas, Depts. of Elect. Engineering, Anatomy, and Psychology, Vanderbilt Univ., Nashville, TN 37240.

The region of the Second Somatosensory Representation, SmII, was mapped with microelectrodes in seven (7) adult grey squirrels, Sciurus carolinensis. Detailed results led to the following conclusions. (1) SmII is located immediately caudal to the representation of the head in SmI (for the organization of SmI, see Nelson and Sur, Anat. Rec. 187: 666, 1977), in an architectonic field previously described as P1 (Kaas et al., J. Comp. Neur. 145: 273, 1972). (2) The contralateral body surface is systematically represented with SmII with the head and face most medial, adjoining the supra-orbital region of SmI. The neck, arm, trunk, leg, and tail are progressively more lateral within SmII. Thus, the head-to-tail progression in SmII is basically medio-lateral rather than rostro-caudal as has been reported for other mammals. (3) The dorsum of the trunk is represented along the caudal margin of SmII and the ventrum is more rostral. (4) Although receptive fields on the glabrous skin of the hand or foot were large and included parts of the arm or the leg, it was possible to conclude that the representation of the hand and foot are located in the rostral parts of the arm and leg region of SmII. Thus, the orientation of the body surface within SmII differs from that usually described, but is similar to that reported for the cat by Haight (Brain Res. 44: 483, 1972).

In each experiment, the rostral border of SmII was defined by extending rows of recording sites into SmI. Similarly, parts of the lateral and caudal border of SmII were delimited by recording sites activated by auditory stimuli. Electrode penetrations were considered to be within SmII when they conformed to a second systematic representation of the contralateral body surface and were related to a body part which was also represented more rostrally, in SmI. Using similar light cutaneous stimulation, receptive fields for comparable body parts were usually significantly larger for recording sites within SmII than for those in SmI. Under recording conditions of urethane anesthesia, receptive fields were predominantly confined to the contralateral body surface. However, a few receptive fields were bilateral and these were continuous across the midline.

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1581 REINNERVATION OF GLABROUS SKIN FOLLOWING NERVE CRUSH - A SINGLE FIBER STUDY IN BABOONS. J.K. Terzis* and R.W. Dykes, (Spon: J.C. Fentress,) Department of Physiology and Biophysics, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The left ulnar nerve was crushed at the level of the wrist in five female baboons (Papio anubis) using sterile surgical techniques. The contralateral unoperated ulnar nerve served as the control. The animals were allowed to survive from one to five months. At monthly intervals the functional properties of the regenerating mechanoreceptive afferent fibers reinnervating the glabrous skin on the ulnar side of the previously denervated hand were investigated and compared to fibers from the control side. The electrophysiological techniques employed in this study included electrical stimulation of the ulnar nerve at the wrist, above the level of the crush, along with single fiber dissection of ulnar nerve at the mid-arm level. This arrangement allowed rapid isolation and identification of a large number of single fibers. Once the latency of the afferent fiber was recorded, the hand was searched for its receptive field. Once identified, this was demarcated under magnification and drawn on enlarged photographs of the hand. Areas of highest sensitivity were outlined. The response characteristics of the normal mechanoreceptive fibers innervating the glabrous skin were used as a standard for comparison to data from afferent fibers reinnervating the contralateral hand. More than 800 single fibers were isolated from the ulnar nerve in approximately equal numbers from the normal and from the injured nerves. The results demonstrated that on the side of the injury, (i) conduction velocities of the nerve fibers which had been separated from their end organs were significantly slower than the controls, (ii) receptive field size and shape, and areas of highest sensitivity, were abnormal in recently reinnervated receptive fields, (iii) the response threshold and stimulus-response relation were altered, and (iv) there was a slow progression of reinnervation with time occuring in a proximo-distal direction. These results can explain some of the observations concerning altered sensibility in the hand following blunt peripheral nerve injuries.

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1582 DIFFERENTIAL SENSITIVITY TO VARIOUS SENSORY STIMULI IN INTACT AND SPINAL HIBERNATING GROUND SQUIRRELS. Delphi M. Toth. Dept. Anat., Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.

In hibernating mammals, conduction in peripheral nerves persists at body temperatures as low as 1°C. In contrast, nerves of non-hibernators cease to conduct at temperatures below 25°C. Hibernating animals, therefore, have the potential to perceive sensory input. Though various authors have briefly mentioned hibernators being disturbed by a variety of isolated stimuli during experiments concerning other aspects of hibernation, no systematic attempt has been made, to date, to determine to which sensory modalities hibernating animals are able to respond and to what degree they are sensitive to specific stimuli within different modalities. Thus, in the present series of experiments, responsiveness to a variety of modalities of sensory stimulation was compared in intact and spinal hibernating Citellus lateralis. Spinal hibernators were prepared by transection of the cord at the level of C_1 during hibernation and were maintained in the cold with artificial respiration. Modalities of stimulation included tactile, thermal, nociceptive, vibratory, proprioceptive, auditory, alimentary and olfactory. Indices of response included occurrence of at least one burst of muscle action potentials, increases in heart rate, respiratory rate and thoracic temperature, occurrence of visible movement, and initiation of an evoked arousal. The occurrence of bursts of muscle action potentials appeared to be the most sensitive index of responsiveness. In all instances this was also the first response of the hibernator following stimulation.

Intact and spinal hibernating ground squirrels alike were most responsive to tactile and nociceptive stimulation, as well as to locally applied vibration. Intact hibernators were strikingly more responsive than spinal hibernators to lowering of the ambient temperature and vibration of the testing chamber. Neither intact nor spinal animals responded to warming or cooling of a small area of the skin, or to the proprioceptive stimulus of tilting the cage. In modalities for which the spinal hibernators were not tested, intact hibernators were unresponsive to auditory stimulation and most responsive to alimentary stimulation. Smelling salts and ether were effective olfactory stimuli but extracts of vanilla and anise were not.

In summary, the hibernator will respond, to varying degrees, to a diversity of peripheral sensory stimuli. It is also evident that the various measures of responsiveness are not equally sensitive as indices of subtle changes in the hibernator's level of arousal.

1583 ORGANIZATION OF POSTCRUCIATE NEURONS WITH RESPECT TO SOMATIC MODALITY AND RECEPTIVE FIELD IN AN AWAKE-RESTRAINED CAT. A.L. Towe and J.C. Slimp. Dept. Physiol. & Biophys. (SJ-40), Univ. of Wash. Sch. of Med., Seattle, WA 98195.

Observations from barbiturate preparations form the primary basis for our ideas about cerebral organization. How well these ideas apply to the unanesthetized condition was examined in a cat equipped for chronic extracellular recording by studying the location, size and modality of the somatic receptive fields of neurons within a 20 mm^2 circular area of postcruciate tissue over a seven-month period. In a sample of 542 neurons obtained from 64 parallel electrode tracks (3 with no neurons and 3 with only one), only 391 were responsive to somatic stimulation. Some topographic organization was evident, with neurons having trunk receptive fields located caudomedially and those having forelimb fields located laterally. However, there was extensive overlap and mixing of fields and modalities in all tracks in which more than two neurons were isolated. In the extreme case, neurons with hindlimb receptive fields were occasionally found intermingled with neurons having receptive fields on the wrist, paw, and/or digits of the contralateral forelimb. Often, two or more neurons were recorded simultaneously; in terms of a superficial vs. deep modality partition, the probability of finding mixed modalities was one-fourth, whereas the probability of finding non-overlapping receptive fields between neurons of the same modality was one-third. For sequentially recorded pairs of neurons isolated at increasing distances apart, the probabilities slowly changed in the direction of increasing disorder. As shown in the following table, the changes were slight.

Separation in mm	Modality similarity			Receptive field overlap*		
	Superfic.	Deep	Mixed	Complete	Partial	No
0	.31	.43	.25	.53	.18	.29
0.25	.32	.29	.39	.44	.32	.24
70.4	.29	.29	.43	.25	.25	.50

*Overlap calculated for pairs of the same modality

When calculated in terms of four submodalities (touch, hair, MTJ, and joint only), the probability that a neuron showing one modality will be followed by another of the same modality was slightly higher than chance, suggesting a minor degree of submodality ordering in depth. Consistent with the idea of topographic organization, receptive field location showed greater ordering in depth. However, the data do not show the high degree of spatial organization with respect to location, size and modality of neuronal receptive fields that one is led to expect from previous studies on anesthetized preparations.
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1584 COMPARISONS OF PUNCTATE, EDGE AND SURFACE STIMULATION OF SLOWLY ADAPTING SOMATOSENSORY AFFERENTS OF CATS. Charles J. Vierck, Jr. Dept. Physiol., Sch. Med., Univ. of North Carolina, Chapel Hill, N.C. 27514, on sabbatical leave from the Dept. Neuroscience, Univ. of Fla., Col. Med., Gainesville, Fla. 32610.

Action potentials of single peripheral afferents were isolated by dissection of the posterior femoral cutaneous nerve or of dorsal roots supplied by this nerve. Units were characterized in terms of adaptation rate, regularity of discharge, temperature sensitivity, responsivity to skin stretch and receptive field (RF) configuration. Afferent fibers from types I and II slowly adapting (SA) mechanoreceptors were selected for stimulation with a servo-controlled stimulator that reliably delivered indentations of 0.09, 0.4 or 0.8 mm. For each of these intensities, the number of action potentials elicited from 0 to 0.1 or from 1.0 to 1.5 sec after stimulus onset was related to the configuration of the stimulus probe in an unequivocal fashion. For all units of both types, a point stimulus centered on the small uniform RF of a type II unit or on a type I dome produced the highest rate of discharge, and indentation by a flat surface over the entire RF produced the lowest rates. For the flat surface probe similar low rates of discharge were produced by 0.09 and 0.8 mm indentations. In contrast, the rate at 0.8 mm was more than double that produced by 0.09 mm when the point stimulus was used. At all intensities and for both sampling periods, stimulation of a dome or a type II receptor by a probe which placed an edge on the RF elicited discharge frequencies that were intermediate to those obtained with the point and the flat surface. These results suggest that the SA units respond best to local deformations. This could provide a peripheral selectivity for edge as well as point detection. Supported by Grants NS 10321 and NS11132 from NINCDS.

1585 THE EFFECT OF DORSAL COLUMN STIMULATION ON THE NOCICEPTIVE RESPONSE OF DORSAL HORN CELLS. Zsuzsanna Wiesenfeld, Ulf Lindblom,* and Daniel N. Tapper. Dept. Physical Biology and Section of Physiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Single dorsal horn cells with nociceptive input were studied in urethane anaesthetized flaxedilized cats. The cats were spinalized at L_1 and the cells were recorded in S_1 segment. Most cells were located in Rexed's laminae IV and V; a few were in the marginal lamina I. The majority of the cells received convergent input from low threshold mechanoreceptors as well as nociceptors responding to noxious heat. The effect of single and repetitive electrical stimulation of the ipsilateral dorsal columns on the activity of these cells was examined. The antidromic response of the afferents contributing to the dorsal columns was monitored by recording from the posterior femoral cutaneous nerve. The intensity of the dorsal column stimuli used was typically twice the threshold required to activate the largest diameter fibers.

A single shock to the dorsal columns usually caused short latency orthodromic activation of the cells, followed by inhibition lasting about 100 msec. The orthodromic activation was not, however, necessary, for the inhibition to occur. The stronger the cell was discharging, the shorter was the duration of the inhibition. Prolonged repetitive stimulation at 3Hz had no effect on the cells' ongoing activity or responsiveness to mechanical or painful stimulation. Repetitive stimulation for 3 or 6 minutes at 50Hz had no effect on about 2/3 of the cells. The rest of the cells were less responsive for up to 30 minutes after the cessation of stimulation. These results may indicate that the population of dorsal horn cells receiving nociceptive input is heterogeneous and various subclasses may have different functions in various types of pain.
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1586 ALTERATIONS IN THE NEUROPIL OF THE THALAMIC VENTRAL TIER NUCLEI IN THE RAT FOLLOWING LESIONS IN THEIR PRINCIPAL AFFERENT INPUTS.
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Based on the delineation of the ventral tier nuclei by McAllister, et al. (this vol.), electron micrographs of the neuropil of the ventral posterior (VPL) and ventral lateral (VL) groups of nuclei were analyzed for population density and volume proportion of synaptic terminals. Because of the relative simplicity of the neuropil of VPL and VL in the rat, synaptic terminals can be grouped into three general categories: 1. small terminals with round vesicles and asymmetric densities (type S); 2. large terminals with round vesicles, asymmetric densities and often a partial glial capsule (type L) and 3. terminals of widely varying sizes that contain flat or pleomorphic vesicles (type F). Alterations in the normal neuropil were determined following lesions of the dorsal column nuclei (DCN), the deep cerebellar nuclei (Cb) and the sensory-motor cortex which are the principal afferent inputs to these thalamic nuclei.

Eighty percent of VPL terminals were type S, 10% were type F and slightly less than 10% type L. The volume of type S and type L terminals was approximately equal. Some type S terminals were of cortical origin and synapsed on small dendrites. The type L terminals were primarily largely of DCN origin and synapsed on proximal dendrites and cell bodies. These observations confirm the results of others. Some type L terminals did not degenerate following nearly complete DCN lesions, and only 20-25% of the type S terminals degenerated following extensive cortical lesions.

Although the population density of terminals is approximately equal in VPL and VL, VL contains many more type F terminals and fewer type L terminals. Cerebellar lesions cause about half of the type F terminals (7-10% of the total number of terminals) in VL to degenerate. Thus, the majority of synapses in both VPL and VL seem to originate from sources other than their principal afferent inputs. We will attempt to correlate these data with a similar analysis of the central intralaminar nucleus currently in progress.

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1587 PHYSIOLOGICAL ORGANIZATION OF THE RAT CORTICAL BARREL FIELD FOLLOWING NEONATAL VIBRISSAL DAMAGE.
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Normal anatomical arrangements of cortical barrels are disrupted by neonatal damage to the mystacial vibrissae in mice and rats. The present experiments were carried out to determine if modified physiological projections accompany these morphological alterations. Vibrissal follicles in rows B and D or row C alone were cauterized in rat pups on the day of birth. When these animals were at least 120 days old the contralateral cortical barrel field was extensively mapped under urethane anesthesia with tungsten microelectrodes. All brains were sectioned tangentially, parallel to the layer IV barrelfield.

Vibrissae did not regrow in 75% of the cauterized follicles. The gaps left on the face by the missing vibrissae were occupied by down hairs. In many cases there were no corresponding gaps between the cortical projections from the intact vibrissal rows. Thus, when row C was missing the projections from row B were contiguous to those from row D. Sometimes there were narrow silent zones where the missing vibrissae would normally project. Most of the vibrissae which regrew in cauterized follicles did not activate any cortical neurons. Cortical areas that did receive projections from such regrown vibrissae were much smaller than normal. Cortical areas for the intact vibrissae adjacent to cauterized follicles increased in size, but these increases were not equal to the size of the normal projection area for the cauterized row.

Nissl-stained sections of these brains showed that in all cases there were five rows of morphologically distinct barrels. Cauterization of vibrissal follicles in one or more rows did not eliminate the corresponding row(s) of cortical barrels. These barrel rows may be reduced in size, but there does not appear to be a significant expansion of the barrels for the intact vibrissae into the territory of barrels for cauterized vibrissae. These experiments show that the increases in the cortical projection areas for intact vibrissae do not result primarily from increases in the size of their morphologically defined barrels. Rather, these results suggest that subsequent to neonatal vibrissal damage in rats, some of the cortical neurons in the corresponding barrels may become activated by projections from adjacent vibrissae.

1588 NATURAL LOSS OF MILK TEETH CAUSES DEGENERATION IN BRAIN STEM.
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Contrary to usual notions, recent studies show that degeneration of central processes occurs after lesions to their peripheral branches and preliminary results reported here indicate further that central degeneration may occur as a natural process. Tooth pulp extirpations in adult cats result in transganglionic axonal degeneration in the spinal trigeminal nucleus (Westrum et al., 1976 Brain Res., 101:137). Therefore since resorption of deciduous teeth causes degeneration of the peripheral axons innervating the teeth (Karlsson et al., 1974 J. Dent. Res., 53: 1428) then exfoliation of milk teeth might be expected to produce transganglionic degeneration in the brain stem trigeminal nuclei. Thus far 8 young cats between 3-9 months of age, and at various stages of exfoliation of deciduous and eruption of permanent teeth, are being used to study the possible degeneration patterns in the brain stem. Animals are perfused with buffered formalin and frozen sections are made in the transverse plane throughout the brain stem to include the entire trigeminal nuclear complex. Serial sections (1 in 12) were stained with reduced silver methods for degenerating axons and terminals (Nauta-Gygax and Fink-Heimer). By 6 months of age, when the milk teeth are actively exfoliating, axonal degeneration can be seen bilaterally in the spinal trigeminal nucleus. The distribution is principally in the ventral part of pars interpolaris and adjacent rostral pars caudalis, near the level of obex and may be more dense on one side than the other. Rostrally pars oralis and the main sensory nuclei are free of degeneration. The amount of degenerative material is reduced and eventually disappears in older animals. The pattern and distribution of the degeneration is similar to that observed in adult cats, 11-14 days after total unilateral pulpectomies that likewise decreases in amount with time. The results show that transganglionic degeneration of central sensory processes does occur in brain stem as an apparent consequence of the natural process of tooth replacement (resorption, exfoliation and eruption) and that the CNS representation for at least part of the innervation of the milk teeth may be similar to that of mature tooth pulps. Also remodelling or reinnervation by the central sensory processes may be occurring along with the changes in peripheral innervation during tooth replacement especially since the degeneration disappears after exfoliation and reappears in the same area following pulpectomies in adults. (Aided by NIH Grants NS09678 and NS04053. The author also acknowledges the technical assistance of Bruce F. Miller, Ph.D.).

1589 PERSISTENCE OF MOTION SENSE AFTER JOINT RECEPTOR BLOCK
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Joint receptors have been considered to be the primary and possibly exclusive mediators of position and motion sense until recently. Recent reports have forced a reconsideration of this view and drawn attention to the possible role of muscle receptors as well. In the past, claims have been made that occlusion of the blood supply to the hand or anesthetic block of the finger joint abolished position and motion sense in the joint. We have devised a procedure that permits motion sense threshold to be continuously monitored for long periods of time so that the effects of occlusion or anesthesia could be followed during various phases of the altered condition. The finger joint (metacarpophalangeal joint of the index finger) was articulated sinusoidally (at 0.5 Hz in most cases). The intensity (amplitude) of the sinusoidal stimulus was slowly increased until the subject felt the motion, whereupon the closure of a switch by the subject caused the intensity of the stimulus to decrease. When the subject no longer felt the motion the switch was opened and the stimulus intensity again increased. This is similar in principle to the von Békésy audiometric technique.

When the upper arm cuff and wrist cuff were inflated (250 mmHg) the motion sense threshold gradually increased from the normal value of about 1° to a point where motion sense was completely lost. When the arm cuff was removed and the wrist cuff was left in place, motion sense returned as reflected by a threshold of about 3°. Blood flow was monitored by use of plethysmographic techniques, insuring complete blockage of flow to the hand. Pin pricks, von Frey hairs, blunt objects and tuning forks were used to assess the degree of loss of sensation in the hand. Motion sense returned even when the hand remained completely anesthetic after removal of the upper arm cuff.

Similar differences between normal and digital nerve block conditions were observed. In this case the first interphalangeal joint was used. 8-10ml 2% Xylocaine was injected to create a ring block proximal to the joint. Again, sensory assessment insured loss of sensation in the vicinity of the joint after nerve block.

These results are taken as support of the hypothesis that joint receptors are not the exclusive mediators of motion sense. Receptors in muscle or tendons must contribute as well.

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1590 THE THALAMOCORTICAL SYSTEM OF THE RAT SOMATIC SENSORY CORTEX: ORGANIZATION, DEVELOPMENT, AND CONNECTIONAL SPECIFICITY.
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Autoradiographic and axonal degeneration fiber tracing studies demonstrate that thalamocortical fibers entering the first somatic sensory cortex (SI) of the adult rat are: (i) distributed mainly to layers VI, IV, and I; (ii) found exclusively in those parts of SI which contain dense aggregates of layer IV granule cells, not in the surrounding and intervening, less granular (but commissurally connected) parts; (iii) distributed in column-like clusters, 250-450 μm in width, throughout the entire extent of the granular aggregates in layer IV and in the subjacent parts of layer VI.

Thalamocortical fibers reach the presumptive layer IV early in cortical development, towards the end of the third postnatal day. The adoption of a generally mature pattern of distribution occurs before layer IV becomes clearly distinguishable from the supragranular layers, before the granule cell aggregates appear, and about 3-4 days before the commissural fibers reach the less granular parts of SI.

The potential interaction between developing commissural and thalamocortical fiber systems was examined by removal on the first or second postnatal day of either the commissural system (by hemidecortication or commissurotomy) or the thalamocortical system (by thalamotomy). In neither case did the laminar or spatial organization of the remaining afferent fiber system show any abnormality when examined experimentally in the mature animal. These findings suggest that both the thalamocortical and commissural fibers specifically form connections with a certain cell class at a particular depth in the cortex, even in the absence of the terminals of a nearby afferent fiber system. The connectional specificity seen in the neocortex contrasts markedly with the plasticity of afferent connections in the allocortex.

In the absence of thalamic input, the cortex shows a slight (13%) reduction in the combined thickness of the granular and supragranular layers. The granule cell aggregates develop to the same horizontal extent as in the normal. Within them, however, subsidiary groupings, such as the "barrels", fail to acquire their definitive form, indicating that only this finer aspect of cortical cytoarchitecture is dependent upon thalamic connectivity.

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1591 RESPONSE PROPERTIES OF SUBSTANTIA GELATINOSA NEURONES IN THE CAT.
Tony L. Yaksh, Patrick D. Wall* and Eugene G. Merrill*. Dept. of Anatomy, University College London, England.

The substantia gelatinosa rolandi (SG) contains many small cells and is penetrated by primary afferents as well as the dendrites of more deeply lying cells. This region may function as an important link in the primary afferent pathway. Yet, the uniformly small size of the cells has made it difficult to record their response properties. With the use, however, of glass coated, platinum-plated, tungsten microelectrodes, we have been able to record reliably the responses of 308 cells located in the SG of the cat. Animals were rendered decerebrate, or decerebrate-spinal while body and cord temperature, blood pressure and arterial pO_2 were monitored and maintained throughout the experiment. Two search procedures were used: 1) the electrode was inserted and spontaneously active (SA) cells were examined (161 cells); 2) penetrations were made while the adjacent Lissauer tract (LT) was stimulated at 1 Hz. In this latter procedure, 56 cells were antidromically driven (followed with a fixed latency of 0.5 - 3 msec) while 91 cells were orthodromically driven (variable latency of 2 - 5 msec and failed to follow at 2 - 10Hz). As the general response properties of cells obtained by either procedure were similar, it appears likely that either approach was sampling from the same cell population. Three unusual types of cell were observed. 1) Small field cells. These units had RFs up to 1.5 cm^2. Often clusters of these cells having small overlapping fields could be noted. We excluded the liklihood that these cells were afferent fibres by virtue of their SA, variable latency upon electrical stimulation of the RF and their inability to follow high frequency stimulation. Penetration through these cell clusters often revealed deeper cells with larger RFs which encompassed the RF of the more dorsal cells. 2) Habituating cells with variable RF field size. 43 units showed marked habituation. In the extreme, a single brush failed to elicit a second response if tested in less than 5 - 10 sec. In several units, the RF appeared to have a variable portion which showed marked transient habituation and a second portion which showed little habituation. 3) Prolonged discharge cells. We observed 56 cells in which a single brush produced stable discharges lasting for several seconds to several minutes. Barbiturates blocked the prolonged discharge. The great majority of cells responded to brush or brush touch and pressure but 59 cells required heavy pressure or pinch of their RF before responding. No cells were detected which responded only to unmyelinated afferents.
(This work was supported by funds from the NIH and MRC).

1592 IDENTIFICATION OF UNMYELINATED FIBERS IN THE TRIGEMINAL MOTOR ROOT OF HUMAN AND CAT.
Ronald F. Young and Richard T. Stevens*. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210

Recent work has focused on the fiber spectrum of spinal ventral roots in various animals. Particular interest has been paid to the identification of unmyelinated fibers since the ventral spinal roots are considered to be primarily motor in function while unmyelinated or C fibers are commonly thought of as being sensory in nature. Specifically, unmyelinated fibers have long been related to the perception of pain. Although unmyelinated fibers have been identified in significant numbers in spinal ventral roots at different segmental levels, there as yet has been no similar analysis of the fiber spectrum of the trigeminal motor root.

In this study, trigeminal motor roots from cat and human autopsy material were fixed and embedded for electron microscopy. Whole root fiber counts of myelinated fibers were obtained by light microscopy of thick sections. Electron microscopy was employed to obtain fiber ratios of myelinated and unmyelinated fibers. The spectrum of fiber diameters in each individual root was determined by the use of a computerized digitizer. Using this method each fiber to be measured was traced with a stylus. This value was automatically entered into a computer for calculation of fiber diameter, computation of histograms and statistical analysis when desired.

The results reveal the presence of unmyelinated fibers in the trigeminal motor root of both species. Such fibers have not previously been reported. A considerable variation in the ratio of myelinated to unmyelinated fibers exists between animals but consistent ratios are present within each species. In the human trigeminal motor root unmyelinated fibers comprise approximately 24% of the total fiber population while in the cat trigeminal motor root the unmyelinated fibers comprise approximately 11%. These results are similar to those reported for spinal ventral roots.

Classical physiological concepts consider the spinal ventral roots and trigeminal motor root to relate exclusively to motor function. The finding of unmyelinated fibers in spinal ventral roots and our confirmation of their presence in the trigeminal motor root has cast considerable doubt on this hypothesis. The findings suggest a partial sensory function for "motor" roots. The occasional failure of trigeminal sensory rhizotomy to provide effective relief of facial pain and the relatively frequent later recurrence of pain will be discussed in relation to the unmyelinated fibers demonstrated in the trigeminal motor root.

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SPINAL CORD

1593 DISTRIBUTION OF ^{3}H NOREPINEPHRINE AFTER LOCAL INJECTION OF ^{3}H DOPAMINE PRECURSOR INTO THE SPINAL CORD OF THE CAT. John L. Alderman, Jewell L. Osterholm, John D. Irvin, Lucas J. Martinez*. Dept. Neurosurgery, Thomas Jefferson Medical College, Phila., Pa. 19107.

Although radiolabelled neurotransmitter precursor loading of the brain for turnover and metabolism studies can be attained through intraventracular injection with relative ease, precursor loading of the spinal cord through comparable routes (cisternal or lumbar) is much less efficient. To circumvent this problem for spinal norepinephrine (NE) studies ^{3}HDA (10uci/10ul) was injected stereotaxically into the medullary portion of the thoracic (T8) cord at the rate of 1ul per minute. After 60 minutes the injected cord region was removed, divided into grey and white matter and cut into 2mm subsections along the cord axis. The ^{3}HNE and ^{3}HDA precursor was then isolated and separated by conventional methods and estimated by liquid scintillation spectrophotometry. Endogenous NE levels for calculation of the specific activity (SA) were determined radioenzymatically. While only 11% of the total cord ^{3}HDA and 12% of ^{3}HNE was found in the white matter compared to 89% and 88% for the grey, the specific activity of ^{3}HNE in the two regions were 40 and 60% respectively, because of the greater endogenous NE concentrations found in the grey. By 60 minutes, ^{3}HDA, was 31% converted to its ^{3}HNE product in the white, and 40% in the grey. Thirty-six percent of the total radioactivity (^{3}HNE + ^{3}HDA) was confined to the first 2mm of injection, and the remainder descended logarithmically for at least another 8mm above and below the injection site. The data suggests that the thoracic axons take up and metabolize endogenously administered precursor nearly as efficiently as the nerve endings. Furthermore, local injections of ^{3}HDA precursor appears to be the route of choice for the study of ^{3}HNE turnover and metabolism in the spinal cord.

1594 THE DORSOLATERAL FUNICULUS OF THE SPINAL CORD: A MAJOR ROUTE FOR DESCENDING BRAINSTEM CONTROL. Allan I. Basbaum and Howard L. Fields, Depts. Anat. and Neurol., UCSF, San Francisco, CA. 94143.

Previous anatomical studies have emphasized that ventral spinal pathways are the predominant locus of brainstem-spinal axons. Recent autoradiographic analysis (Basbaum et al, PNAS, 1976), however, has demonstrated a significant medullospinal projection in the dorsolateral funiculus (DLF). To more precisely determine which brainstem cell groups contribute axons to dorsal vs. ventral spinal pathways, the present study was undertaken.

The thoracic and lumbosacral cord was exposed in barbiturate-anesthetized cats. Large bilateral injections (40μl) of a 50% solution of horseradish peroxidase (HRP) were placed in the L6-L7 lumbar spinal segment. At the same time, partial spinal cord lesions were made at upper thoracic levels. The lesions resulted in selective interruption of retrograde transport of HRP from the lumbar cord to the brainstem and thus delineated the pathways by which brainstem neurons project to the spinal cord. The spinal lesions included: unilateral DLF; bilateral DLF; dorsal hemisection; ventral hemisection; lateral hemisection plus contralateral section of the ventral and ventrolateral funiculus. In the latter, the dorsal columns were sectioned bilaterally, thus isolating one DLF. Retrograde transport of HRP was demonstrated with hydrogen peroxide and o-dianisidine according to the technique of de Olmos.

Cell bodies with axons in the ventral and ventrolateral funiculi are located in the medial and lateral vestibular nuclei. Scattered cells were also found in medullary reticularis paragigantocellularis, gigantocellularis, pontis caudalis and oralis. Raphe pallidus and obscurus project primarily via ventral pathways as do a few cells in the locus caeruleus.

The most striking observation was the large numbers and wide distribution of brainstem neurons contributing axons to the DLF. These include cells in the ipsilateral hypothalamus, Edinger-Westphal nucleus, and large numbers in subcaeruleus and reticularis magnocellularis (lateral to the raphe magnus). Paragigantocellularis, locus caeruleus, parabrachialis, retroambiguus and the solitary nucleus also send axons in the DLF, some of which course in the contralateral DLF. Numerous cells in the raphe magnus, but far less in raphe pallidus contribute axons to the DLF. Cells of the red nucleus (pars magnocellularis and parvocellularis) and a discrete paralemniscal region of the pontine reticular formation send axons exclusively in the contralateral DLF. These data underscore the importance of the dorsolateral funiculus in transmitting a profound and diverse source of brainstem modulation of spinal neurons. Supported by NS-70777, 11529, 05272, 11614.

1595 ROLE OF DORSAL RADICULAR ARTERIES IN RECOVERY OF FUNCTION AFTER DEAFFERENTATION. A.J. Berman, A. Marty* & A.S. Rothman*. Veterans' Administration Hospital, Bronx, N.Y.

Although considerable purposive movement remains after section of the dorsal roots serving the forelimbs in the monkey, there is much variation between animals in their postoperative status. Not only are there differences in rate and degree of recovery of movement sequences with the affected forelimbs but, in addition, some animals develop weakness of the hindlimbs while others do not. The hindleg weakness, when it occurs, may appear immediately after surgery or may develop slowly during the postoperative period.

It has been reported that preservation of the radicular vessels embedded within the root fibers results in more rapid recovery of function than when they are sectioned during dorsal rhizotomy. The present study was undertaken to test whether either the degree and rate of recovery of forelimb function or the maintenance of hindlimb function was related to the degree of interference with the dorsal radicular blood supply to the cord during surgery. Fourteen M. mulatta were subjected to bilateral dorsal rhizotomy C2-T3. In seven cases, the dorsal radicular arteries were spared while in the other animals the vessels were sectioned with the roots. This phase of the surgery was performed under 16 and 25 power magnification.

Weakness of the hindlimbs occurred in two of the seven animals in which the vessels were sacrificed but was not present in any of those in which the vessels were spared. As far as forelimb function was concerned, however, no differences between groups were noted.

All animals were perfused with micropaque solution at the time of sacrifice and x-ray examination of the vasculature of the spinal cord was performed. Those animals which developed weakness of their lower extremities were found on histological examination to have degeneration in the pyramidal tracts.

Results of this study indicate that sacrificing the dorsal radicular arteries during C2-T3 dorsal rhizotomy does not contribute to the degree of neurological deficit in the forelimbs consequent to the deafferentation, but may result in long tract involvement, thus affecting function well below the level of the lesion.

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1596 EFFECT OF PARACHLOROPHENYLALANINE ON BLOOD FLOW IN THE TRAUMATIZED SPINAL CORD. Robert A. Brodner and Robert H. Roth. Sect. Neurosurg., Dept. Surg. and Dept. Neuropharm., Yale Sch. Med., New Haven, Conn. 06510.

In recent studies (Br Res 118: 348, 1976) we have demonstrated a significant correlation between elevated cerebrospinal fluid (CSF) serotonin and reduced intramedullary blood flow following experimental spinal cord injury. In an effort to increase post-traumatic spinal cord perfusion and, thereby, inhibit ischemic changes, serotonin was depleted by pretreatment with parachlorophenylalanine (PCPA), a serotonin antagonist, and the resultant effect upon intramedullary blood flow was studied.

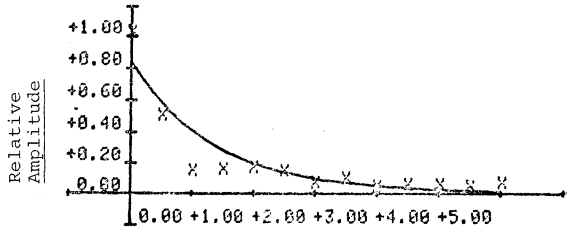
Thirty adult cats were pretreated 48 hours prior to the experiment with PCPA (400mg/kg) and then their thoracic spinal cords were contused with a 500gm-cm force. Control animals were traumatized at the same injury level, but received no PCPA. At various time intervals up to 1 hour after trauma, CSF serotonin concentrations were assayed and the intramedullary blood flow patterns were studied by the thioflavine S fluorescent technique.

A statistically significant decrease in CSF serotonin was noted in all the PCPA treated cats as compared to the untreated controls. The post-traumatic reduction in spinal cord blood flow seen in the control group was reversed in the treated animals to a state of normal perfusion.

In this study, prevention of the post-traumatic rise in CSF serotonin by PCPA has been correlated with improved microcirculatory dynamics within the traumatized spinal cord, demonstrating that the decreased blood flow and attendant ischemia associated with spinal cord injury may be amenable to pharmacological therapy.

1597 TRANSMITTER TURNOVER IN THE MONOSYNAPTIC SPINAL PATHWAY OF MAN. A.A. Buerger and B. Sherman*. Depts. of Physical Med. & Rehabilitation and Physiology, California Coll. of Med., Univ. of California, Irvine, CA 92717.

In an attempt to determine the rates of release and replenishment of the transmitter involved in the monosynaptic spinal reflex, we have studied the exponential decay of the H reflex and the M reflex in the gastrocnemius-soleus of man due to trains of 16 stimuli. Trains were delivered at rates ranging from 0.25 Hz to 5 Hz and the areas under the M and H responses were calculated. At any one frequency for four trains the average area of each of the 16 M and H responses was calculated and exponential decay curves were fitted to the 16 averaged areas. (See example below):



The rate of decay of the H reflex (as well as the rate of decay of the ratio between the H and M reflexes) increase with the frequency of the stimulus train. This decay probably is due to homosynaptic depression and the parameters of the exponential curve can therefore be used to calculate the rate of transmitter turnover in the monosynaptic spinal pathway of man. We have done this, using the methods of Esplin et al (e.g. J. Neurophysiol. 40:95-105, 1977). An analysis of the available data suggests that both release and replenishment of the transmitter for this monosynaptic reflex are linearly related to stimulus frequency and that, at comparable frequencies of stimulation, the values we have calculated for humans are of the same order of magnitude as those which have been obtained in other vertebrates.

1598 NONLINEAR ANALYSIS OF DORSAL HORN CELLS. Arthur D. Craig, Jr. and Daniel Tapper, Dept. of Physical Biol. and Section of Physiol., N.Y.S. Coll. Vet. Med., Cornell Univ., Ithaca, NY 14850

In order to overcome limitations in the use of the Poisson impulse train variant of the Wiener nonlinear analysis method, due in part to axonal refractory periods, a new technique has been devised for the dynamic analysis of neural pulse-input/pulse-output (nonlinear) systems. By utilizing an input pulse train which is a Bernoulli series of (geometrically distributed) intervals, it has been shown that a nonlinear system may be modeled by a set of orthogonal functionals similar to the Poisson-Charlier functionals, and whose kernels may be estimated by cross-correlation. Following implementation on a PDP-11/05-GT40, the technique has been applied in the spinal cat, using input trains of varied mean and modulus. Input pulses are used to electrically elicit action potentials in single afferent fibers innervating Type I cutaneous receptors (Haarscheiben, HS). Responses of monosynaptically connected lamina IV-V dorsal horn cells (DHC) are recorded extracellularly, and digitilized as interval sequences. The HS-DHC system kernel estimates are presently computed up to the second order, and the model responses to single pulses (linear kernel) and pulse pairs of several interstimulus intervals plotted. The latter compare favorably to PST's generated by the classical condition-test paradigm. Inspection of model output suggests dynamic system dependence on mean input pulse rate, as well as other indications of third or higher order system characteristics. For example, the inhibitory period often observed following the monosynaptic response can deepen and occur with shorter latency as the input mean is increased, even to the point of occluding any preceding facillitatory period. Examples of late facilitation (15-100 msec) have also been observed for close pulse pairs, where the classical two-pulse PST gave little indication of such a phenomenon. Compared to the classical paradigm, this technique acquires more information characterizing a neural system in less time, and, further, is readily adapted to multi-input and cascaded systems. Nonlinear system models will be used to generate generalized models of DHC operation.

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1599 COMPENSATORY CHANGES IN MOUSE SCIATIC NERVE FOLLOWING CONTRALATERAL NERVE DAMAGE. Richard A. Gerren*, Douglas E. Groswald*, and Marvin W. Luttges. Aero. Eng. Sciences, Univ. of Colorado, Boulder, CO 80309.

Neurophysiological and neurochemical analyses were used to study the changes in mouse sciatic nerves as a consequence of damage to the contralateral sciatic nerve. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis separations of whole sciatic nerve proteins revealed transient changes in relative protein concentrations of the undamaged nerves. Such changes occur within one week after damage to the contralateral nerves. During the same time period electrically-elicited responses in the undamaged nerves differ from responses of control nerves tested in mice which had sustained no nerve damage. The experimental nerves exhibit considerable changes in relative refractoriness when tested with paired electrical stimuli of variable interstimulus intervals. Spinal influences are implicated in these altered response characteristics since removal of such influences by proximal nerve sectioning results in loss of the effect. Transpinal alterations in excitability and electrophoretic alterations in protein composition are both consistent with compensatory spinal changes induced by contralateral nerve damage.

1600 PROJECTIONS AND ORIGINS OF THE SPINOTHALAMIC TRACT IN THE RAT. Glenn J. Giesler, Jr., Allan I. Basbaum and Daniel Menétrey*. Dept. Psych., UCLA, Los Angeles, CA 90024 and Depts. Anat. and Physiol., UCSF, San Francisco, CA 94143.

In an earlier study (Giesler et al., Brain Res. 118: 320, 1976), antidromically identified spinothalamic tract (STT) neurons in the rat were found to be widely distributed throughout the dorsal horn and intermediate gray zone of the lumbar enlargement. Interestingly, it appeared that STT cells located within the intermediate zone with non-cutaneous receptive fields tended to be activated only from midline thalamic structures. At the same time, STT neurons encountered within the dorsal horn having response properties characteristic of that area were activated uniformly from lateral thalamus. To pursue this apparent dichotomy of thalamic afferents, we have used the retrograde transport of horseradish peroxidase (HRP) to assess projections and origins of the STT.

Injections of .05 to 0.1μl were made in various thalamic areas. Retrograde labeling of STT neurons was examined following treatment with either diaminobenzidine or o-dianisidine (J. de Olmos) reaction techniques.

HRP injections into the intralaminar group produced labeling which was restricted to the intermediate zone and ventral horn at all spinal levels. STT cells were found bilaterally only in upper cervical segments. A discrete grouping of neurons was located in the ventromedial aspect of the dorsal horn in segments L3-6. A similar but less dense pattern of labeling throughout the cord was achieved following injections into more anterior medial thalamic areas (medial dorsal n., central lateral n.).

Injections filling the ventral posterior lateral n. produced a strikingly different distribution of STT neurons. In upper cervical segments, particularly dense groups of cells were found in the caudal extension of the dorsal column nucei. Many neurons were also found within the lateral white matter which appear on cellular morphological grounds to be the lateral cervical n. Less dense labeling was produced in C1-3 in the ventral horns bilaterally. In the cervical enlargement and thoracic cord, HRP-positive cells were found almost exclusively in the dorsal horn. Labeling in the lumbar cord was also encountered within the dorsal horn, including the ventromedial area of L3-6 identical to that labeled by medial thalamic injections.

Together with our earlier findings, these data suggest that in the rat the STT carries cutaneous and non-cutaneous somatic information separately in pathways having different cells of origin and terminal projections.

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1601 THE FILUM TERMINALE OF THE FROG SPINAL CORD: GABA UPTAKE BY A NON-TRANSFORMED GLIAL PREPARATION. S. Glusman, M. Pacheco* and B. Haber, Depts. of Physiology and Cell Biology, Centro de Investigacion del IPN, Mexico, and the Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

In the tadpole, the tail is innervated by motor neurons present in the terminal segments of the spinal cord. During development leading to metamorphosis, the tail is resorbed, and the majority of the neuronal elements involved in its innervation degenerate. The resulting structure after metamorphosis is the frog filum terminale (FT); this is that portion of the spinal cord which extends caudally beyond the level of the last spinal root. The frog FT has been shown to consist of a few motor neurons, some descending fibers, relatively few ependymal cells and to a large extent glia. The glia are the predominant cell type in the FT, and have astrocytic like morphology. We have studied the regional distribution of GABA uptake in the frog spinal cord, in comparison to the filum terminale, as well as in the spinal cord of the developing tadpole. AOAA (10^{-4}M) was included in the incubation medium, and better than 95% of intracellular radioactivity is present as GABA. The uptake of GABA in the frog spinal cord is mediated by a saturable, high affinity, Na^+ dependent mechanism. As one proceeds caudally beyond the level of the last spinal root, the velocity of the GABA uptake increases markedly, and in the caudal portion of the FT it is three times higher than in any other portion of the frog cord. A similarly increased uptake in the FT is also observed for glycine, taurine and ß-alanine. In the tadpole during early stages of development, GABA uptake is similar in all segments of the spinal cord. However, during the later stages of development, GABA uptake in the most caudal portion of the tadpole cord increases dramatically, and approaches the rates seen in the FT of the adult frog. Frog FT segments incubated with ^{3}H-GABA ($2X10^{-7}$M) were fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer, and sections were prepared for light and electron microscope radioautography. The radioautographic studies clearly show extensive labelling of the glial cells, as well as labelling of the ependymal cells. The latter however represent only a very small percentage of the overall FT cellular population. In summary, the frog FT represents a unique non-transformed glial preparation which can be used to advantage to study the accumulation of neuroactive amino acids and other properties of normal glia.

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1602 THE EFFECTS OF PROGRESSIVE HYPERCAPNEA ON SPONTANEOUS POLYNEURONAL ACTIVITY IN THE CHICKEN EMBRYO SPINAL CORD. T. J. Gonya* and B. T. Stokes (SPON: E. K. Michal). The Ohio State University, Columbus, Ohio 43210.

Neurogenesis of the motor neuropil in the chicken embryo is characterized by peak frequency in multiunit spontaneous activity (bursts) at about day 13, which subsequently decline until day 19 of development. Several lines of evidence have suggested that the emergence of active inhibitory neural circuits is responsible for this modulaton of polyneuronal activity. The effects of changes in respiratory gases (PO_2, PCO_2) on spontaneous neural activity in lumbosacral spinal cord have been investigated late in chick development (14 to 19 days). Polyneuronal burst frequency was monitored while five minute pulses of altered gaseous environments were applied to the embryonic environment. The effect of an initial CO_2 pulse has been studied and reported previously. 3,1, or .5% CO_2 has been shown to depress activity at 14 and 16 days. However, in 19 day old embryos, .5% CO_2 actually enhances burst activity; while 1 and 3% CO_2 pulses will still inhibit ongoing activity.

In addition to the above, data has now been collected on the effects of multiple hypercapneic episodes on burst activity. Consequtive pulses of 3, 1, or .5% CO_2 appear to produce less of an effect on polyneuronal discharges when compared to an initial pulse. Low doses of CO_2 may transiently increase neural activity between pulse periods (.5% CO_2 was seen to enhance activity during a second pulse). However, with one exception, burst frequency was seen to decline significantly throughout subsequent interpulse periods.

The data suggest that hypercapnea does influence the polyneuronal activity patterns recorded in chicken embryo spinal cord. The response is clearly dependent on concentration and age of the ambryo, and may be related to the blood gas changes which occur throughout normal development.

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1603 SPINAL PROJECTIONS FROM THE DORSOLATERAL PONS-LOCUS COERULEUS AND ADJACENT RETICULAR FORMATION-A LIGHT AND ELECTRON MICROSCOPIC STUDY. G.E. Goode, J.W. Atkinson*, G.F. Martin and K.A. Crutcher, Dept. Anat., Sch. Med., The Ohio State University, Columbus, Ohio, 43210.

Following horseradish peroxidase injections into lumbar, thoracic or cervical spinal cord, reaction product can be localized in neurons of the rostral pons, specifically, the locus coeruleus and locus coeruleus pars alpha and in the adjacent reticular formation of the North American opossum. As in other mammals the catecholamine content of these neurons can be made to fluoresce. Autoradiographic labelling was plotted in the spinal cord following injections of [^{3}H] amino acids that cover the locus coeruleus, locus coeruleus pars alpha and adjacent reticular formation. In cervical segments, label was found over axons in the white matter. These labelled axons form a continuous peripheral band that arches from the dorsolateral fasciculus to the ventral median fissure. These axons were separated from the external limiting membrane by unlabelled axons. Terminal label was found along the reticulated periphery of the gray matter and overlapped the location of fluorescent varicosities seen in the spinal cord. A similar pattern of degenerating axons was found in cervical and lumbar cord two days after stereotaxic lesions which destroyed part of locus coeruleus or an area just medial to the nucleus. A band of degenerating myelinated axons (3-15µ in diameter) extended from the anterolateral quadrant, around the periphery of the ventral funiculus and well into the septomarginal white matter. Degenerating terminals could not be found in spinal gray following survival times of 48 hours, although degenerating axons and preterminal fragments were identified. Degenerating terminals have thus far been identified on intermediate and distal dendritic shafts in the marginal neuropil of the ventral horn as early as 20 hours after rostral pontine lesions. Rapid degeneration of myelinated axons in cervical and lumbar spinal cord following brainstem lesions has not been generally recognized.

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1604 EXPERIMENTAL SPINAL CORD TRAUMA: PATHOPHYSIOLOGY OF THE ACUTE STAGE. Patricia A. Grady, Otis R. Blaumanis*, John T. Lucas*, S. Kumar Jirge* and Erland Nelson. Dept. of Neurol., Sch. Med., Univ. of Maryland, Baltimore, Md. 21201.

The pathophysiology of acute experimental spinal cord injury was studied in mongrel dogs lightly anesthetized with nitrous oxide in oxygen. The spinal cord was exposed by laminectomy and traumatized at the C_2 level by dropping a weight (600-1200 gm. cm. impact). Blood pressure, heart rate and respiratory rate were monitored during the experiments. Animals were maintained on a respirator following the trauma. Dogs were perfused through the heart with 2.5% buffered glutaraldehyde immediately, and at one, two and six hours after injury. Spinal cords were removed and prepared for light, transmission (TEM) and scanning electron microscopy (SEM).

Apnea and bradycardia occurred immediately after spinal cord injury. An increase in systolic and diastolic blood pressure which occurred within seconds persisted for several minutes.

Gross observation revealed moderate to severe hemorrhage on the dorsal surface of the cord as well as in the underlying gray matter at the site of injury. This hemorrhage was usually not apparent or minimal when animals were perfused immediately after the injury. Correlative SEM demonstrated thrombus formation in dorsal and anterior spinal arteries. Patchy endothelial desquamation with platelet aggregates as well as frank thrombosis were observed. Consistent hemorrhagic non-perfusion was observed in areas of gray matter underlying the site of injury. Thrombi were also observed in intraparenchymal vessels.

These observations suggest that the cardiovascular and respiratory responses described occur as a direct result of the initial impact to the spinal cord and that thrombi and vascular occlusion occur secondary to direct injury to blood vessels. Vascular injury may play a primary role in the pathogenesis of traumatic myelopathy.

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1605 GLUCOCORTICOID EFFECTS ON SPINAL CORD FUNCTION. Edward D. Hall, Thomas Baker and Walter F. Riker, Jr. Dept. Pharmacol., Cornell University Medical College, New York, N.Y. 10021.

The effects of intensive glucocorticoid dosing on segmental spinal cord function in cats have been studied. Adult cats were treated with triamcinolone (8 mg/kg) intramuscularly once daily for seven days. On the first post-treatment day segmental spinal reflexes in the lumbar cord were examined. Under halothane, a tracheotomy was performed and the spinal cord transected at C1. Anesthesia was terminated and positive pressure ventilation with room air begun. A dorsal laminectomy exposed segments L4 to S2. The popliteal fossa of one leg was dissected to expose peripheral nerves for stimulation. All reflex responses were recorded at ventral root L7. Complete neuromuscular paralysis was maintained with gallamine.

As a result of the triamcinolone pre-treatment, polysynaptic discharge was significantly enhanced. Single monosynaptic (2N) transmission at 0.2 Hz was increased, but not significantly. Paired stimuli disclosed that the rate of 2N recovery after single impulse transmission was greatly improved in glucocorticoid animals. Consistent with this was the fact that the 2N pathway in treated preparations was better able to maintain repetitive transmission at 50 Hz. 2N Post-tetanic potentiation was enhanced, and was seen at conditioning frequencies which barely evoked PTP in untreated animals. Direct and recurrent post-synaptic inhibitory mechanisms were not affected, but presynaptic inhibition was greatly increased. Consistent with this increase was an increase in the amplitude of the dorsal root potential V.

The intravenous administration of a single large 90 mg/kg dose of the water-soluble glucocorticoid methyl prednisolone brought about a striking elevation in the amplitude of the 2N response. This occurred within 20 seconds after injection, with no effect on polysynaptic discharge. Increased spindle discharge as a contributing factor was ruled out. Low doses of Na pentobarbital (2.5 to 10.0 mg/kg) prevented the 2N increase.

These results reflect a direct action of glucocorticoid on the primary afferent terminals improving excitability and transmitter release. These effects may in part explain the reported benefits of glucocorticoid treatment in acute spinal cord injury (Ducker, T.B. and Hamit, H.F., J. Neurosurg. 39 (1969) 693-697).
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1606 EFFECTS OF INHIBITION ON FIRING RATES OF HOMONYMOUS MOTONEURONS OF DIFFERENT TYPES. Dale A. Harris and Elwood Henneman. Dept. Physiology, Harvard Medical School, Boston, Mass. 02115.

Single motor axons from plantaris or medial gastrocnemius motoneurons were isolated in ventral root filaments of decerebrate cats. After collection of 10-12 units, their critical firing levels (CFLs) were measured one after the other in a short time. Pairs of units of similar size as judged by their CFLs were placed on separate recording electrodes and their responses to repetitive shocks applied to the muscle nerve were compared simultaneously. The rates of each unit were determined by averaging during the first second of discharge following the onset of stimulation. By varying the intensity or frequency of repetitive stimuli, the two units could be made to respond with a wide range of firing rates. It was found that the ratio of the rates of the two simultaneously recorded units was approximately constant over the entire range of measured rates. Next, with the excitatory input maintained at a constant level that elicited maximal firing rates, stimuli were applied to the nerve of an ipsilateral antagonistic muscle to produce graded inhibition of the firing rates of the test units. As wide a range of inhibited response rates as possible was obtained by varying the parameters of the inhibitory input. Ideally, this range of inhibited rates was comparable to that obtained by varying the excitatory input alone. It was found that the ratio of the rates of inhibited pairs were not always constant over the entire range of measured rates. Detailed inspection of the data showed that, in general, the ratio of rates of pairs of motoneurons of similar size (CFL) and firing rate (ratio appro. = 1) remained constant when the degree of excitation or inhibition was varied, indicating that the cells were receiving inputs of comparable effectiveness. However, although the ratio of rates of units of similar size (CFL) but radically different rates (ratio approx. = 2) remained constant when excitation was varied, the ratio changed considerably when inhibition was varied, indicating that the units were not equally affected by the inhibition. Further tests revealed other differences between units of equal size, which depended on the similarity or dissimilarity of their firing rates. All results were in agreement with previous findings indicating that there are at least two types of motoneurons in a single pool having different physiological response characteristics.

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1607 DELAYED 'OFF-RESPONSES' IN THE SUBSTANTIA GELATINOSA. Ian Hentall. Dept. of Biology and Research Lab. of Electronics, M.I.T., Cambridge, MA 02139.

Platinum-tipped, metal microelectrodes registered, in the substantia gelatinosa of unanesthetized spinal cats, single units (34) which responded to gentle mechanical stimuli with a 5-20 sec. burst of impulses, 50-100 msec after withdrawal of the stimuli, but which were mainly silent before and during stimulation. A second touch during bursts inhibited firing, with the onset time indicating that this effect came from Aα-fibers. Noxious thermal and mechanical stimuli were initially ineffective, although, over a period of some minutes, a regular background activity (1-5 impulses/sec.) developed following such stimulation. In units possessing this activity, a noxious stimulus increased the firing rate during its application and produced a nearly silent period in about the first sec. following its removal.

Cutaneous electrical pulses delivered every second, a repetition period exceeding burst length, enabled an average-response histogram to be constructed which showed that the process giving rise to the initial delay resembled in time course the negative dorsal root potential (DRP). This process is regarded as an extension of Aα inhibition. Based on these data, a direct synaptic effect upon the afferent telodendron from the inhibitory process in these units' dendrites is suggested as one cause of the negative DRP.

1608 SPONTANEOUS ANTIDROMIC ACTIVITY IN SENSORY AFFERENTS. S. G. Kamerling and E. G. Anderson. Univ. Ill. Med. Ctr., Chicago, IL 60612.

Recent studies from this laboratory have demonstrated the occurrence of spontaneous, antidromic action potentials traveling in the dorsal roots of unanesthetized spinal cats (Repkin et al., Brain Res. 117: 147, 1976). The present studies show that this activity, termed the dorsal root discharge (DRD), occurs in a select population of fibers. Although the DRD was observed in S1 dorsal roots, in cutaneous nerves, and in peripheral muscle nerves (flexors and extensors) of de-efferented spinal cats, it seems confined to larger diameter afferent fibers. Conduction velocities of single-action potentials of the DRD were estimated from the time taken for such potentials to travel between two pairs of bipolar hook electrodes which supported the nerve. Spike conduction velocities were distributed among three groups with means of 38 ± 1.3, 72 ± 1.7, and 96 ± 2.4 meters/sec (± S.E.M.), respectively. These values correspond to Group I or Group II afferents. The majority of fibers showed conduction velocities within the second group.

These data indicate that antidromic activity can be detected in selected populations of sensory afferents, even under conditions of minimal surgical intervention. The physiological significance of this activity is not yet apparent, but we have observed it to be markedly decreased by small doses of pentobarbital (2 mg/kg, iv) and profoundly facilitated by GABA (100 mg/kg, iv). This latter effect is presumed to result from the GABA-induced depolarization of primary afferents. Since previous studies have shown that the DRD was not depressed by bicuculline or semicarbazide, the DRD apparently reflects non-GABA-mediated, tonic primary afferent depolarization. (Supported by USPHS Grant NS 12649.)

1609 *LOCALIZED UPTAKE OF ^{14}C 2-DEOXYGLUCOSE IN CAT SPINAL CORD AND DORSAL COLUMN NUCLEI IN RESPONSE TO SCIATIC NERVE STIMULATION.* John S. Kauer and William B. Stewart*. Depts. of Neurosurgery and Physiology, *Yale Univ. Sch. of Med.*, New Haven, Conn. 06510.

2-deoxyglucose(2DG) has been successfully used to map increased metabolic rate related to neuronal activity in various CNS structures in response to physiological stimulation(Kennedy et al., Science.187:850,1975; Sharp, Br. Res. 100:46,1976; Sharp et al., Br. Res. 98:596,1975; Sokoloff, in Brain Work, 1975 p385). It has also been used to demonstrate increased activity in the dorsal horn of rat lumbar spinal cord(SC) with electrical stimulation of the sciatic nerve(Kennedy, ibid.,1975). These latter results are of particular interest because they suggest that 2DG uptake can reflect neuronal activity elicited by artificial(electrical)stimulation. In addition, the uptake patterns are consistent with known projections of the sciatic nerve demonstrated with other methods(Sprague and Ha, Prog.Br. Res.11:210,1964). The present study was designed to examine further the activity produced by sciatic nerve stimulation in the cat, since the larger spinal cord of this animal permits a more precise correlation of 2DG uptake with the anatomy of the SC. Uptake patterns were examined in unanaesthetized control cats and in anaesthetized cats in which the sciatic nerve was stimulated. The autoradiographs of the activity patterns were compared with Nissl stains of the tissue from which they were taken in order to establish the location of the radioactive label.

The SCs in the control cats show the central gray matter to have a greater level of baseline uptake than the surrounding white matter. In those animals in which the sciatic was stimulated with brief shocks at 10 Hz or more, there was localized uptake in the ipsilateral dorsal horn in lumbar segments 5-7 and in the ipsilateral nucleus gracilis. Animals stimulated at 2 Hz showed no stimulus-related uptake. In those sections showing maximal uptake, the activity was localized to Rexed's laminae 3-5, with less uptake in laminae 1 and 2. In sections rostral and caudal to the level of maximum uptake, the activity was limited to a strip extending across laminae 3-6 on the medial margin of the dorsal horn. The recordings of the compound action potential proximal to the stimulation site indicated that the large fibers composing this nerve were maximally activated. There was no apparent A delta or C fiber activation. In those sections showing ipsilateral dorsal horn activity, there was no obvious contralateral uptake nor increased focal uptake in the ipsilateral ventral horn, although motoneurons in this region were presumably activated antidromically. These patterns of uptake, localized to the dorsal horn and dorsal column nuclei, are consistent with activation of and 2DG uptake in the synaptic terminals of large afferent fibers.

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1610 *POST-TETANIC POTENTIATION AND DEPRESSION OF RENSHAW CELL DISCHARGES.* K. Krnjević and D. Lekić*[1]. Dept. of *Anaesthesia Research, McGill Univ., Montreal H3G 1Y6.*

In cats under Dial anaesthesia, tetanic stimulation of a lower lumbar ventral root (at frequencies of 50-500 c/s, for 5-20 s) evokes after a variable early period of depression, a very prolonged potentiation (PTP) of Renshaw cell discharges in response to single shock stimulation of ventral root. This is seen as an increase in the number of spikes per discharge (reaching a maximum of +20-50 % after tetani at 200-300/s) rather than a rise in the peak frequency of firing - indeed this was usually reduced. As elsewhere the magnitude and duration of PTP are a function of frequency and duration of tetanus. The PTP can last for > 3 min and is occasionally followed by a late phase of depression. There is a comparable PTP of the spontaneous firing of Renshaw cells. On the other hand, tests of Renshaw cell responsiveness to ACh show a very pronounced post-tetanic depression that may block all responses for 10-20 s, complete recovery being delayed for 1-2 min after a 10 s tetanus at 100-200/s. This post-synaptic change, which is evident after tetanic stimulations at frequencies as low as 10/s, must contribute to the overt early post-tetanic depression of firing, and probably significantly limits the maximum observable PTP. It cannot be mainly caused by desensitization to ACh, since discharges evoked by aspartic acid are also markedly diminished after a tetanus. It is not clear whether this reflects a general, non-specific post-activity depression, or a prolonged reciprocal inhibition of Renshaw cells. The former seems to be indicated by the marked reduction in spontaneous firing that immediately follows strong excitation with ACh: the absence of subsequent potentiation of spontaneous firing shows that PTP is a presynaptic phenomenon, as previously found at other junctions.

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1611 THE EFFECT OF OSMOTIC PRESSURE ON REFLEX ACTIVITY IN THE ISOLATED FROG SPINAL CORD. D. A. Lake and C. D. Barnes. Dept. of Physiology, Texas Tech Univ. Sch. Med., Lubbock, Tx. 79409.

Furshpan (J. PHYSIOL. 134: 689, 1956) reported that with an increase in the osmotic pressure of the external fluid there was an increase in MEPP frequency but no change in the size or quantal content of evoked EPPs in the isolated frog nerve-muscle preparation. The present study was done to determine if osmotic agents had a similar lack of effect on evoked activity in the central nervous system. Spinal cords of Rana Pipiens were isolated and superfused with a modified glucose-Ringers solution (NaCl, 100 mM; KCl, 2.5 mM; Na_2HPO_4, 2.5 mM; NaH_2PO_4, 0.45 mM; $CaCl_2$, 1.9 mM; $NaHCO_3$, 12 mM; and glucose, 2.8 mM) aerated with a 95% O_2-5% CO_2 gas mixture (pH 7.4). The tenth dorsal root (DR 10) was supramaximally stimulated and ventral root reflexes (VRR) were recorded in VR 10 and VR 9. Increasing the osmotic pressure of bathing medium with mannitol produced a concentration-related depression of bath intra- and intersegmental VRR. The VRR depression was only partially reversible at all concentrations of mannitol added. This response will be further characterized by the use of other agents (urea, glucose, glyerol) which are known to penetrate the cell membrane at various rates.

1612 RHOMBENCEPHALIC CELLS OF ORIGIN OF DESCENDING SPINAL SYSTEMS IN THE ATLANTIC STINGRAY. R.B. Leonard and W.D. Willis. Marine Biomedical Inst. and Depts. of Anatomy and Physiology & Biophy., University of Texas Medical Branch, Galveston, Texas 77550

There have been few experimental studies of the cells of origin of descending spinal cord pathways in non-mammalian vertebrates. We have begun to investigate these systems in the stingray using the retrograde transport of horseradish peroxidase (HRP) to avoid the problems in recognizing chromatolytic reactions in these animals, particularly in small neurons. HRP was injected into the spinal gray using a microelectrode. The approach was through the dorsal columns to minimize direct injury to fibers to caudal spinal levels, and 0.2 µl was slowly injected in each of several tracks. Initial animals received multiple bilateral injections, while subsequent animals received more restricted unilateral injections with a contralateral hemisection rostral to the injection site. In addition, current experiments are examining the possibility of a somatotopic organization within these systems. Following injections at caudal pectoral fin cord levels, labelled cells are found within the spinal cord and continuing through the spino-medullary transition zone dorso-lateral to the central canal. These appear to be long propriospinal neurons continuing into the caudal reticular formation. A distinct group of neurons replaces the somatic motor column as it becomes smaller throughout this zone. In addition a few labelled cells are located dorsal to the central canal where the solitary complex joins on the midline. There is an extensive projection from the raphe beginning at medullary levels and continuing rostrally past the inferior olive. Some raphe cells are located ventrally between the olives, but the majority are situated dorsally. Additional cells are located adjacent to the raphe through most of this region. Another group of cells are located dorso-lateral to the rostral pole of the olive and continue rostrally a short distance. There are scattered cells among the VIth nerve rootlets which increase rostrally as a distinct group ventral and slightly medial to motor V. Scattered cells are among the rootlets of the anterior lateral line-vestibular nerve complex as well as within the rostral pole of the posterior lateral line lobe. We assume most or all of these are vestibulo-spinal neurons. Another group of relatively small cells is located laterally at the level of the cerebellar peduncles. The descending systems from the caudal brainstem in the stingray appear similar to those described in mammals. However, we have refrained from assuming homologies of many of these groups until more is known about the organization of and afferents to each group. Supported by NS 11255, NS 05434 and a grant from the Muscular Dystrophy Association of America.

1613 MONOSYNAPTIC REFLEX CONNECTIONS OF EXTERNAL URINARY AND EXTERNAL ANAL SPHINCTER MOTONEURONS. Robert Mackel* (SPON: B.W. Peterson) Rockefeller Univ., New York, N.Y. 10021.

The striated muscles of the pelvic floor which are directly related to the continence aspect of micturition and defecation are the external urethral and external anal sphincters (EUS, EAS). The most important source of motor supply to the two external sphincters is derived from the deep branches of the mixed somatic pudendal nerve.

To study the properties of motoneurons supplying the sphincter muscles experiments were performed on male cats anesthetized with chloralose, using intracellular recording techniques. The pudendal nerve branches to the EUS and to the EAS were dissected out and mounted separately for antidromic and orthodromic stimulation. The motoneurons which were identified antidromically and localized by dye injection were found to be intermingled in the S2, S3 segments of the spinal cord. The conduction velocity of the EUS and EAS motor axons ranged between 50-70 m/sec.

Monosynaptic connections to EUS and EAS motoneurons were demonstrated upon stimulation of the S_2-S_3 dorsal roots and upon stimulation of the same nerve branch which contained the motor axon. The central latencies (central conduction time plus synaptic delay) as measured from the arrival of the afferent volley at the cord dorsum to the beginning of the EPSP ranged between 0.6 and 0.9 msecs. Such short latency EPSPs were observed in 13 out of 17 cells with dorsal root stimulation and in 13 out of 25 cells with stimulation of the nerve branch containing the motor axon. The monosynaptic response could in each case be evoked at low threshold and only from the homonymous nerve branch.

These results support the observations of Garry et al. (1959) and Bishop et al. (1956) who observed a significant increase in tonic EMG-activity and a powerful contraction upon distension of the EUS and EAS in the cat. The results of the present experiments suggest that a monosynaptic reflex similar to that present in other somatic muscles contributes to the stretch-evoked contraction of the EUS and EAS. Such reflexes in the EUS and EAS presumably play a direct role in preventing the emptying of the bladder or the rectum. Preliminary results show that EUS and EAS motoneurons also receive direct inputs from descending spinal pathways.

Bishop, B., Garry, R.C., Roberts, T.D.M., Todd, J.K.: J. Physiol. (1956), 134, 229-240.
Garry, R.C., Roberts, T.D.M., Todd, J.K.: J. Physiol. (1959), 149, 653-665.

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1614 DIFFERENTIAL PROJECTIONS OF CAT MEDULLARY RAPHE NUCLEI IN SPINAL CORD WHITE MATTER. Richard F. Martin, Larry M. Jordan and William D. Willis. Marine Biomedical Institute, Dept. Physiol. & Biophy. and Anat., Univ. Texas Med. Br., Galveston, TX. 77550 and Dept. Physiol., Univ. Mannitoba, Winnipeg, Canada.

Serotonin terminals found in the spinal cord gray matter have been attributed to descending projections of the medullary raphe nuclei. Although some previous studies in the cat indicate that fibers from the nucleus raphe magnus course in the dorsolateral fasciculus, it was uncertain what the origin is of the many 5HT axons projecting in ventromedial and a ventrolateral fasciculi. This study shows that fibers coursing in these regions arise from the nuclei raphe pallidus and obscurus.

Neurons of medullary raphe nuclei of the cat were retrogradely labelled following injections of horseradish peroxidase (HRP) into the lumbar enlargement (L6). A 50% solution of HRP (1.0-5.0 µl) was injected bilaterally into the spinal cord gray matter of anesthetized animals using a glass micropipette. Partial cordotomies were performed in these animals at the time of injection to restrict transport of HRP to those axons projecting in the spared white matter. The cordotomies were performed at T12-L1, a level separated from the HRP injection site by four to five segments to avoid HRP uptake from cut axons.

In three cats in which dorsolateral cordotomies were performed, a large percentage of neurons of both nucleus raphe pallidus and nucleus raphe obscurus were labelled following HRP injection. No neurons in the rostral half of nucleus raphe magnus were labelled in these animals. In two cats in which ventral cord sections were performed, HRP labelled occurred in large percentage of neurons in the rostral half of nucleus raphe magnus. A reduced number of labelled cells were found in nucleus raphe pallidus or nucleus raphe obscurus in these animals.

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1615 PROPERTIES OF SYNAPTIC LINKAGE FROM "DISTANT" AFFERENTS ONTO DORSAL HORN NEURONS. L.M. Mendell, E.M. Sassoon*and P.D. Wall* Cerebral Functions Group, University College London, Gower St. London, U.K.

These experiments were prompted by recent findings that dorsal horn neurons in lumbar segments of cat spinal cord (e.g. L7) can be driven by electrical stimulation of a distant dorsal root (e.g. L3 or L4), but not by natural stimulation of skin supplied by these roots. We placed 9 pairs of 25 gauge needles in the skin of the limb and the flank for electrical stimulation of the skin ($A\beta$-δ strength), and studied the responses of dorsal horn neurons in the L7 segment with cutaneous receptive fields. Extracellular recordings in decerebrate-spinal preparations revealed that the probability of a unit responding to stimulation of the flank (innervated by L3 or L4) increased as the receptive field was more proximal (i.e. "hip" units responded with a higher probability than "toe" units). Generally, the repetitive discharge was weaker and more variable in response to flank stimulation than to stimulation within the receptive field on the limb. These differences were less evident when the natural receptive field was proximal (i.e. on the leg or hip). Intracellular recording with citrate electrodes in both decerebrate-low spinal and anesthetized-low spinal preparations revealed that an even larger proportion of L7 neurons was excited, often subliminally, by flank stimulation. Each cell responded (probably monosynaptically) with a short latency to stimulation within the receptive field even at high frequencies (e.g. 50 Hz). In contrast, the response to flank stimulation usually had a long latency (>10 msec) and was generally erratic even at 1 Hz although in some cells EPSPs followed at 1 Hz or even (in a very small minority) at 10-50 Hz. Increased ability to follow high stimulation frequencies was associated with shorter response latencies (as short as 4 msec) and with more proximal natural receptive fields (leg or hip). In some cells stimulation of the flank yielded IPSPs or EPSP-IPSP sequences which exhibited no set order from cell to cell (i.e. IPSPs could precede, follow or overlap EPSPs). We conclude that a small minority of EPSPs from distant afferents could be monosynaptic but that the projection is primarily polysynaptic. However, the possibility of low efficacy monosynaptic projection failing to follow at 1 or 10 Hz cannot be discounted. Either way, the inability for the response to follow at high frequencies indicates that input from the flank cannot undergo significant temporal summation. This, coupled with the relatively weak input, probably explains why natural stimulation of the flank fails to drive cells in L7. (Supported by NIH, MRC and a Josiah Macy, Jr. Foundation Faculty Scholar Award (L.M.)).

1616 DECENERATION AND EARLY NEURONAL OUTGROWTH FOLLOWING SPINAL CORD TRANSECTION IN XENOPUS LAEVIS TADPOLES. Mary Ellen Michel* and Paul J. Reier. Dept. Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201

Whereas only limited neuronal regeneration appears to occur in the mammalian CNS, several nonmammalian vertebrete species posssess a remarkable capacity for structural and functional recovery following CNS injury. In order to characterize some of the cellular mechanisms underlying such regenerative ability, degeneration and early neuronal outgrowth were examined in transected spinal cords of Stage 54 tadpoles. Following complete cord section at lumbar levels, animals were sacrificed between 1 and 14 days; specimens obtained immediately adjacent to the cut ends as well as from the intervening lesion zone were examined by electron microscopy. Within 2 days numerous swollen axons and distended myelin sheaths were present, and by 5 days Wallerian degeneration was extensive. Very little cellular debris and only a few degenerating fibers were still present adjacent to or within the lesion zone by 14 days. Major cyst formation and glial-connective tissue scarring did not occur during this degenerative period. Concurrent with the massive degenerative changes noted at 5 days was an early outgrowth of fibers into the lesion zone from both rostral and caudal ends. The number of these processes increased dramatically between 5 and 10 days. At 14 days the majority of neuronal profiles in the lesion zone were organized into fascicles surrounded by either cytoplasmic processes or a single basal lamina. Many of the cells associated with these bundles exhibited cytological features similar to those of immature glia found within the normal Xenopus spinal cord. Other cells were derived from the surrounding meninges. Most of the fibers within the initial caudal cord segment were organized into smaller fascicles surrounded by ependymoglial processes. The early post-transection period in this system is thus characterized by rapid degeneration, debris removal and early onset of regeneration without the formation of cysts or glial-connective tissue scars. Furthermore, the presence of cellular sheaths around fascicles of neurites may provide a permissive structural framework for neuronal outgrowth. (Supported by Bressler Reserve Fund Grant from the Univ. Maryland and a Predoctoral Fellowship awarded by the Paralyzed Veterans of America Technology and Research Foundation).

1617 RESPONSES OF VENTRAL HORN CELLS IN THE CAT LUMBAR SPINAL CORD TO CUTANEOUS STIMULATION. Helen H.Molinari and Dan R. Kenshalo. Dept. Psychol., Fla. State Univ., Tallahassee, Fla. 32306

The responses to cutaneous stimulation of cells located in the ventral horn (laminae VII and VIII) of the cat lumbar spinal cord were compared with those of dorsal horn cells (laminae I-VI). Tungsten microelectrodes were used to search the spinal cords of cats anesthetized with chloralose and paralyzed with gallamine triethiodide. Units were classified by stimulating the hindlimbs with hair movement, tactile stimulation with von Frey hairs, thermal stimulation, and noxious stimulation with smooth and serrated forceps, and determining the type of stimulus that produced the largest response. To avoid stimulation of deep receptors, the noxious pinch stimuli were presented while the skin was pulled away from the underlying tissue.

The vast majority of units in the ventral horn responded maximally to noxious stimulation, whereas units were found in the dorsal horn which responded to hair movement, to touch, or to noxious stimuli. Of the ventral horn cells, about half responded only to pinch, while the others responded to pinch and strong tactile stimuli or to pinch and heat.

The nociceptors in the ventral horn tended to have larger receptive fields than the dorsal horn nociceptive, hair, or tactile units. However, there was considerable variability in the sizes of the receptive fields of the ventral horn cells. One unit responded to stimulation of 2 cm^2 of the ipsilateral foot, while another responded to stimulation of any spot on the lateral surface of either hindlimb. Most of the ventral horn cells (75%) had their receptive fields confined to a single area on the ipsilateral or contralateral hindlimb and thus carried relatively localized noxious information. The remaining ventral horn units were activated by pinch over large areas of either hindlimb and provided non-localized information about the impingement of nociceptive stimuli on virtually any part of these limbs. The receptive fields of dorsal horn cells were confined to the ipsilateral hindlimb or back.

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1618 TRAUMA INDUCED CHANGES IN THE SPONTANEOUS ELECTRICAL ACTIVITY IN THE SPINAL CORD OF THE CAT. J.T. Molt, D.A. Poulos and R.S. Bourke, Div. of Neurosurgery and Dept. of Physiology, Albany Medical College, Albany, N.Y. 12208

Hemorrhagic necrosis resulting from blunt trauma to the spinal cord is a progressive destructive process which first consumes gray and then white matter. As the lesion develops, descending modulation of spinal cord neurons caudal to the injury site is interrupted resulting in changes in various potentials which can be recorded from the cord. One such potential is the spontaneous spinal electrogram (SEG). The SEG as recorded from the dorsal spinal gray matter of the cat consists of low voltage background activity (25 μV) upon which occur spontaneous, random, monophasic, larger voltage (50-200 μV) potentials termed negative sharp waves (NSWs) which are 20-40 msec in duration and are recorded as negative going to an indifferent electrode. Recent evidence indicates that the NSWs may be generated spontaneously by the same neurons from which the negative components of the cord dorsum potential can be evoked. It has been demonstrated that generation of NSWs is inhibited by both segmental and supraspinal input. To test the hypothesis that the SEG may be a sensitive indicator of the degree of destruction resulting from a traumatic insult to the cord, groups of anesthetic-free, decerebrate cats were subjected to either a 500 gm-cm, a 250 gm-cm or a 100 gm-cm injury by dropping a 50 gm weight 10, 5, or 2 cm respectively onto the exposed dura of the cord. The percent change in the number of NSWs recorded from the exposed surface of the cord 3 cm caudal to the injury site was plotted as a function of time over a 6 hr period following injury. The slope of the least squares linear regression line correlated positively with the severity of injury measured with a strain gauge at the time of the injury (impulse) and later confirmed histologically (see Table, * indicates significance at 5% level, n = 10).

	r (correlation coefficient)
slope vs impulse	.70 *
slope vs white matter lesion	.74 *
slope vs gray matter lesion	.32
slope vs total lesion	.71 *

These results indicate that the SEG may serve as a sensitive indicator of the degree of spinal cord injury (especially to white matter) and may be useful in the assessment of various treatment modalities used to reverse the effects of trauma. Supported by NIH grant NS 13042.

1619 SPINAL CORD REGENERATION IN RATS FOLLOWING IMMUNOLOGICAL SUPPRESSION AND INDUCED TOLERANCE TO CNS ANTIGENS. Kevin R. Nelson*, Earl R. Feringa and H. Lee Vahlsing*. Depts. Neurol. and Path., VA Hosp. and U. of Mich. Med. Ctr., Ann Arbor, MI 48105.

The purpose of this investigation was to determine if an immune response to central nervous system (CNS) antigens plays a role in preventing the regeneration of long descending motor axons within the spinal cord of the rat.

Forty inbred, isogeneic female rats were divided into three study groups. In an attempt to induce tolerance to CNS antigens, 2 groups (11 animals each) received a 75 mg injection of isogeneic spinal cord emulsified in saline on day 1 of life, and then once a week until the spinal cord was transected at 6 weeks of age. In addition, one of these two groups was given cyclophosphamide, 75 mg/kg, 48 hours after spinal cord transection, for immunosuppression. The third group (18 animals) also had spinal cord transections at 6 weeks of age and served as untreated controls.

Three months were allowed for neural fibers to regenerate. Two methods were used in all animals to detect regeneration. First, the spinal cord was stimulated proximal to the transection site, and signals picked up from dorsal and ventral rootlets distal to the transection. For each animal, 128 such stimulus response curves were averaged by a computer and results evaluated in a blind fashion for possible axonal transmission across the transection site. Evidence for transmission across the transection was found in none of the controls, in 2 receiving spinal cord injections alone ($p=.042$), and in 2 receiving spinal cord and cyclophosphamide injections ($p=.042$).

The second evaluation method utilized anterograde axonal transport of tritiated proline which had been injected into the sensory-motor cortex 21 days before the animals were sacrificed. Identical measured lengths of spinal cord taken from above and below the transection site were solubilized and placed in scintillation fluid. The number of disintegrations per minute (dpm) for each segment was recorded and the value dpm distal/dpm proximal was calculated for each animal. The mean value for the group receiving spinal cord injections alone was .066 and did not differ significantly from the control mean of .069 ($p=.392$). However, the mean of .127 for the group receiving spinal cord injections and cyclophosphamide did differ significantly from controls ($p=.037$).

The electrophysiological evidence suggests that animals made tolerant to brain antigens have an improved ability to regenerate intraspinal axons. The radioisotope study indicates that more tritiated proline was transported to the distal spinal cord segment in tolerant animals which were also treated with cyclophosphamide. This drug may act as an additional immunosuppressant or by another unidentified mechanism.

1620 INCREASE IN Ia-MOTONEURON PROJECTION FOLLOWING SPINAL CORD TRANSECTION. S.G. Nelson and L.M. Mendell. Duke University Medical Center, Durham, N.C. 27710

Individual averaged EPSPs produced by single medial gastrocnemius (MG) Ia fibers in MG motoneurons become significantly larger on the average following spinal cord transection at T13 or L5. These conclusions were obtained by comparing data from spinal anesthetized (Nembutal, 35 mg/kg) cats to those from intact anesthetized cats using the spike triggered averaging technique (Mendell and Henneman, J. Neurophysiol., 34:171, 1971). These amplitude increases were observed following acute transection at either level, but chronically only after the L5 transection. The time course of the increase following L5 transection suggests two separate processes since the initial EPSP amplitude increase was attenuated 5 days after transection and a secondary increase began at about 2 weeks. Following transection at T13, there was an immediate marked increase in average EPSP amplitude, followed by a gradual decline to normal levels over a period of 2-3 weeks. Some variability between animals was observed, presumably resulting from as yet undetermined differences in lesions and/or variability in the projection of Ia fibers (Type X and Type Y, Scott and Mendell, J. Neurophysiol. 39:679, 1976). Increases in mean amplitude were marked by a virtual disappearance of small EPSPs (< 100 μV) and also by the appearance of large (400-1200 μV) brief EPSPs rarely seen in intact animals. The mechanism underlying this EPSP enlargement could be presynaptic (e.g., tonic presynaptic disinhibition) or postsynaptic, although our data indicate no generalized changes in the latter (e.g., motoneuron resistance). Another possibility is a structural change in the Ia-motoneuron synapse (e.g., collateral sprouting of Ia fibers). Consistent with this, single Ia fibers evoked EPSPs in a larger proportion of homonymous and heteronymous motoneurons than normal, particularly in L5 transected preparations. Since normal EPSPs smaller than 15 μV were not resolved, this increase in projection frequency may in some cases have been due to increases in mean EPSP amplitude resulting in fewer small ones being missed. However, higher projection frequencies were occasionally seen in preparations chronically transected at L5 when the Ia fiber produced EPSPs of normal amplitude. The sprouting hypothesis could also account for increased mean EPSP amplitudes following chronic L5 transection (which substantially denervates triceps surae motoneurons) compared to no increase following chronic T13 transection (which produces much less denervation of triceps surae motoneurons). Other mechanisms (e.g., denervation supersensitivity) may also contribute to this phenomenon. (Supported by NS 34680, NS 08411, GM 00929).

1621 RELEASE OF ACETYLCHOLINESTERASE BY CULTURED SPINAL CORD CELLS. T. H. Oh, J. Y. Chyu* and S. R. Max. Depts. Anatomy, Neurology and Pediatrics, Univ. of Maryland Sch. of Med., Baltimore, Md. 21201.

The release of acetylcholinesterase (AChE) from neurons was studied using cultured chick embryo spinal cord cells. Cells dissociated from 12-day-old chick embryo spinal cords were grown in culture for 10-12 days. Numerous well differentiated spinal neurons were found after 7-10 days in culture. AChE activity per dish increased by 60 fold from days 2 to 12. AChE was released into the surrounding media by the cells when they were incubated either in the standard culture medium or the serum-free medium. AChE release was significantly reduced when protein synthesis and microtubules were disrupted by cycloheximide and colchicine, respectively. Histochemical localization of AChE indicated that the synthesis and release of AChE are attributable to neurons. Cultured chick embryo brain and neuroblastoma cells also released AChE into the media. These results are discussed with regard to possible physiological roles for AChE secretion from neurons. Supported by grants from NSF (BNS 76-15370), NIH (NS 11342), Bressler Fund and MDAA.

1622 MORPHOLOGY OF ENDINGS OF PRIMARY AFFERENT COLLATERALS TO THE SPINAL CORD SELECTIVELY STAINED BY ANTEROGRADE MOVEMENT OF HRP Eric Proshansky* and M. David Egger. Dept. Anat., CMDNJ-Rutgers Med. Sch., Piscataway, N.J. 08854.

Analysis of dorsal root axons selectively stained by uniform filling with HRP indicates that this technique can effectively demonstrate, in the adult cat, each of the distinct types of dorsal root collateral described by Cajal and later workers using the Golgi method in fetal and neonatal material. Adult cats were sacrificed after immersion of severed dorsal rootlets for 24 hr in a 40-60% solution of HRP in distilled water. Spinal cord sections processed by the diaminobenzidine technique showed an abundance of dorsal root collaterals homogeneously filled with HRP. Axons or terminals examined in the light microscope showed no evidence of anterograde degeneration. (In animals sacrificed at 48 and 72 hr after rootlet transection, many axons were both swollen and constricted and numerous fragments of HRP-filled material, presumably disrupted axons, were visible.) The study of L5-S1 dorsal root collaterals in 24 hr material indicated that fibers to laminae I-III include both thin axons (<1 μm) entering by way of Lissauer's tract, and thicker fibers entering by way of the dorsal column. These latter fibers enter lamina III from its ventral surface, forming the classically described "flame-shaped" endings within lamina III and the ventral portions of lamina II. In laminae IV-VI, moderately thick (1.5-2.0 μm) collaterals form extensively ramified terminal arborizations having clusters of terminal knobs. The terminal fields of these fibers are distributed across the laminar boundaries and over wide mediolateral portions of the dorsal horn (200-400 μm). Collaterals were also stained within the intermediate region and ventral horn. One group of these fibers formed a dense, circumscribed terminal field laterally adjacent to the central canal. The thickest (4-5 μm) dorsal root collaterals were observed to enter and terminate within the ventral horn. These collaterals, which are likely to be derived from Ia fibers, begin to branch after passing through the dorsal horn, and divide repeatedly within the ventral horn. Terminal knobs 3-5 μm in size are formed at the ends of the branches or, commonly, on long, extremely thin side-collaterals. The terminals of these fibers are distributed throughout much of the ventral gray matter.

The ability to stain previously recognized varieties of dorsal root collaterals in large numbers by HRP filling suggests that this technique will now permit the detailed morphological description of primary afferents in the adult spinal cord.

(Supported by USPHS grant NS 12261.)

1623 THE SYNAPTIC ORGANIZATION OF THE DORSAL HORN OF THE MONKEY SPINAL CORD: A QUANTITATIVE ELECTRON MICROSCOPIC STUDY. Henry J. Ralston, III. Department of Anatomy, Univ. of California School of Medicine, San Francisco, CA. 94143.

In order to determine the contribution of various synaptic types to the population of synapses in the monkey dorsal horn, the fine structure of the horn has been examined in specimens obtained from normal animals and from 8 animals surviving 18 hours to 32 days following labeling of dorsal root fibers, the labeling obtained by extradural dorsal rhizotomy of 3 consecutive dorsal roots of one side, and by the injection of 100μC of H^3 leucine into each of the corresponding dorsal root ganglia of the opposite side. Specimens were processed for conventional electron microscopy (EM) and EM autoradiography (ARG). Vertically oriented sections running from dorsal column white matter through the base of lamina IV (about 800μ total) were scanned, and counts were made of synaptic and other profiles contained in consecutive 50μ regions of the total 800μ length of section. For ARG, both the radioactive and non-radioactive sides of the cord were processed, to check for background silver grains.

The distribution of the several synaptic types varied significantly from lamina I to lamina IV. Of the more than 6000 synaptic profiles classified, round-vesicle (R) synapses accounted for 51%, flat vesicled (F)-36%, and dense-cored (D) vesicled-8%. In laminae I, II and the upper zone of III, R's were about 55-65% of the total, and F's only 13-25%; deeper in III and IV, the number of F's equalled or exceeded those of R's. D's constituted 25% of the total in I, 15% in upper II, and 5% or less at deeper levels. Following rhizotomy, degenerating synapses were first recognized at 2 days survival, reached maximum numbers at 4-5 days, and were uncommon at survival periods greater than 10 days. The number of degenerating synapses totaled from all animals in any lamina never exceeded 6% of the total of normal synaptic profiles: in I-5%; II-6%; III-6%; IV-3%. The ARG results of the distribution of grains seen with fast transport times demonstrated a maximum of 31% of grains found over synaptic profiles, the remainder principally overlying myelinated and non-myelinated axons; thus, fast-transport ARG is not primarily a marker of synapses in the cord. Of the labeled profiles, R's accounted for 10% in I, 22% in II, 28% in III, and 9% in IV; D's were 6% in I; 5% in upper II, and unlabeled at deeper levels; F's were not labeled above background.

Dorsal root axons thus appear to synapse in about equal numbers in laminae II & III, and in lesser numbers in I & IV; they may be classed principally as R's or D's, and participate chiefly in axodendritic and axoaxonal synapses. (Supported by NS-11614 from NIH).

1624 REFLEX INTERACTIONS OF MUSCLES CONTROLLING HEAD POSITION. Samuel Rapoport* (SPON: V.J. Wilson). Rockefeller Univ., New York, N.Y. 10021.

Neural control of limb movement is characterized by a pattern of reciprocal innervation between antagonistic muscles, with supraspinal centers exerting some of their effects by modulating reflex interactions. The present investigation was conducted to explore whether a similar mechanism is involved in the control of head position.

In the cat, sternomastoid and cleidomastoid (SCM) flex the head and rotate it to the opposite side. The ipsilateral biventer and complexus (BC) extend the head and rotate it to the ipsilateral side. Thus SCM and BC are functionally antagonistic muscle groups. The synaptic effects of SCM and BC afferents upon motoneurons of these muscles were studied using intracellular recording. In most cases, cells were polarized to enhance inhibitory effects not apparent at the resting potential. Motoneurons were identified by antidromic stimulation of the muscle nerves. 13 cats were utilized, 10 anesthetized with chloralose, 2 with pentathol, and one decerebrate.

Stimulation of a muscle's own afferents, at strengths near threshold for the afferent volley, produces Ia EPSPs in these motoneurons at a mean latency of 0.8 msec, s.d.=0.5 n=49 (all measurements herein are from positive peak of the incoming volley). When the afferents to the antagonist muscle are stimulated at strengths higher than 1.5 x threshold of the lowest threshold fibers, both EPSPs and IPSPs occur. In SCM mean IPSP latency is 8.7 msec (n=10, range 4.8-12.5) and mean EPSP latency is 2.7 msec (n=10, range 1.5-7.6). In BC mean IPSP latency is 3.8 msec (n=17, range 1.6-7.0), and mean EPSP latency is 2.5 msec (n=4, range 1.2-3.7). The latency measurements of the observed IPSPs, and the stimulus strengths required to elicit them, suggest that, in contradistinction to limb motoneurons, there is no disynaptic Ia inhibition between the antagonists BC and SCM (n=56).

These data, together with the well organized monosynaptic projections to BC motoneurons from brainstem centers, suggest that segmental networks are less important in the control of head position than in the control of limb movements. Rather, the pattern of activity providing control of head position may be imposed upon motoneurons by supraspinal centers.

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625 VISCERO-SOMATIC CONVERGENCE IN THE LATERAL CERVICAL NUCLEUS OF THE CAT. Don Rigamonti, Georgetown Medical School, Dept. of Anatomy, Washington, D.C. 20007.

The spino-cervico-thalamic tract is generally considered a major pathway transmitting signals of an exteroceptive nature to the cerebral cortex. Recently, we reported that the tract carries information from visceral structures. This was suggested by a single unit analysis of the lateral cervical nucleus (LCN), a major relay nucleus for the pathway. Further experiments were conducted to determine if there was convergence of signals from somatic and visceral structures in the LCN.

Alpha-chloralose anesthetized adult cats were operated having pairs of electrodes attached to the splanchnic nerve and thoracic sympathetic chain to deliver graded stimuli and record the resultant neurogram, respectively. Blood gasses and core temperature were monitored and maintained within acceptable physiological ranges. Histological reconstruction of frozen sections stained for Nissl substance allowed reconstruction of spinal cord and midbrain, recording and stimulating, sites. The ipsilateral LCN was searched with micropipettes filled with an electrolyte-dye solution. A hunting stimulus was applied to the contralateral midbrain by an electrode grid and antidromic responses were amplified, displayed and photographed using standard techniques. Units responding to midbrain stimulation were classified as tract cells or neurons intrinsic to the nuclear complex based on the antidromic response. Subsequently, neurons were tested for input from the splanchnic nerve and natural stimulation of hair, joints and deep structures.

Extracellular recordings in the LCN indicate the presence of both tract and intrinsic neurons. We have also confirmed earlier reports suggesting input to the LCN from high threshold myelinated afferents of the splanchnic nerve. The majority of units also exhibited convergence of input from somatic structures. The most effective stimuli were hair movement and pressure applied to deeper structures of the ipsilateral forelimb, trunk and hindlimb. Thus it appears that the tract of Morin serves to integrate exteroceptive as well as interoceptive information.

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1626 INTRACELLULAR ASPECTS OF THE SCHIFF-SHERRINGTON PHENOMENON. J. C. Schadt and C. D. Barnes. Dept. Physiol., Sch. Med., Texas Tech Univ., Lubbock, TX 79409.

The intracellular aspects of the Schiff-Sherrington phenomenon were studied in decerebrate cats. Forelimb motoneurons were identified by antidromic activation from the deep radial, median, or ulnar nerves. Reversible post-brachial spinal cord transection was produced by coldblock while simultaneously monitoring resting potential and input resistance of identified forelimb motoneurons. Motoneurons with axons in the radial nerve showed a decrease in membrane potential and a decrease in input resistance during coldblock. Median and ulnar motoneurons showed an increase in membrane potential and a decrease in input resistance. Several cells were encountered in the region of the motoneuron pool which could not be antidromically identified. The input resistance of these cells always decreased while the cells showed either increases or decreases in membrane potential. We conclude that the Schiff-Sherrington phenomenon is mediated thru facilitation of extensor and inhibition of flexor α-motoneurons.

1627 THE LOCALIZATION OF CORTICAL AND SUBCORTICAL NEURONS PROJECTING TO THE SPINAL CORD IN THE RAT. Jean M. Schoenen* and Valerie B. Domesick. Mailman Research Center, McLean Hospital, Belmont, MA 02178

The purpose of this study was to map the cortical fields which give rise to corticospinal fibers. In addition, neurons in several subcortical areas which project to the spinal cord were identified. Horseradish peroxidase (0.5-5µl of a 13% solution) was injected manually into cervical, thoracic, and lumbar segments of the spinal cord in 10 rats. Following a survival time of 24 hours, the brains were prepared according to the method of Graham and Karnovsky (1966). The labeled cortical neurons were mapped on a dorsal view of the cortex.

Labeled neurons in the cerebral cortex were small, medium, and large pyramidal cells, located exclusively in Layer V. These corticospinal neurons occupy a remarkably large field which extends laterally and caudally farther than previously indicated by various architectonic maps. Injections in the cervical spinal cord labeled neurons in a large field about 3.5mm wide, which begins rostrally in front of the genu of the corpus callosum, and extends caudally to a level just rostral to the splenium. A somatotopic arrangement of corticospinal neurons was evident: the field of neurons labeled by injections at sub-cervical levels did not extend as far rostrally and narrowed progressively in the medial direction. Thus, neurons labeled by lumbar injections were confined to a comparatively narrow medial strip of cortex. When projected upon Krieg's (1946) architectonic map, the field of corticospinal neurons included parts of areas 4 and 6 in the frontal cortex and areas 1, 3, and 7 in the parietal cortex. Two other, smaller fields of labeled cells were identified in the anterior medial cortex and in the lateral cortex just above the rhinal fissure.

Further labeled cells were identified at all levels of the brain stem. In the diencephalon, such cells appeared in the zona incerta and in the hypothalamus, namely the nuclei lateralis, perifornicalis, periventricularis and paraventricularis (parvocellular sector) hypothalami. In the midbrain, labeled cells were identified in the stratum griseum profundum of the superior colliculus, central gray, and the nuclei of Edinger-Westphal, interstitialis of Cajal, raphis dorsalis and ruber. At pontine levels, labeled cells were located in the nuclei tegmenti dorsalis lateralis, locus coeruleus, raphis magnus, and reticularis gigantocellularis . At medullary levels, labeled cells were found in the vestibular nuclei, the raphe region, the nucleus commissuralis, and the lateral reticular formation. That serotonin, noradrenalin or TRH are found in several of these subcortical cell groups may explain the presence of these substances in the spinal cord. (Supported by USPHS Grant MH 25515 and International Research Fellowship No. 5 F05 TW0223502.)

1628 THE EFFECT OF 5-HYDROXYTRYPTAMINE ON FROG MOTONEURONS. Brimmer R. Sherman*, Richard F. Thompson. Department of Psychobiology, UCI, Irvine, CA 92717.

An electrophysiological study was undertaken to elucidate the role of 5-hydroxytryptamine (5-HT) in the amphibian (Rana catesbeiana) spinal cord. Isolated spinal cords were hemisected and maintained in a chamber which had continually circulating amphibian Ringer's solution. The media was oxygenated and maintained at 9° ±1°C. 5-HT in various concentrations was applied to the cord by superfusing it with a Ringer's solution containing the drug.

The addition of 5-HT to the superfusate (10^{-3}-10^{-6} M) produced a transient increase of the lateral column elicited ventral root response (LC-VRR). This increase occurred 3-7 minutes after the application of the drug and was followed by a long lasting depression of the LC-VRR; however, the LC-VRR returned to the pre-drug level following a wash off period with normal media. 5-HT (10^{-3}-10^{-4} M) also produced a reversible depolarization of the motoneurons, as determined by a positive deflection of the ventral root potential, whether the cord was superfused with normal Ringer's or with Ringer's containing 20 mM $MgSO_4$. The LC elicited focal potential recorded from a micropipette in the motoneuron pool can easily be differentiated into pre and postsynaptic components. The presynaptic component corresponds to the orthodromic invasion by the action potential volley into the LC terminals, while the postsynaptic component corresponds to the synchronous discharge of the motoneurons. 5-HT (10^{-3}-10^{-5} M) produced a transient increase followed by a decrease of the postsynaptic component of the LC focal potential, while having no effect on the presynaptic component. The postsynaptic component of the response returned to normal following a wash off period with normal media.

The depolarization of amphibian motoneurons by 5-HT has previously been reported (Comp. Biochem. Physiol. 23:553, 1967); however, this could be produced by the action of 5-HT sensitive interneurons on motoneurons. This was tested by blocking synaptic transmission with high Mg^{++} concentrations. Motoneurons are depolarized by 5-HT in a high Mg^{++} media, indicating that 5-HT acts directly on the motoneurons. Possible implications of these findings will be discussed.

1629 ORIGINS OF THE LONG DESCENDING AND ASCENDING PROPRIOSPINAL PATHWAYS IN CAT AND MONKEY. Robert D. Skinner, Joe Dan Coulter and Allen B. Chatt. Dept. Anat., Univ. Arkansas Coll. Med., Little Rock, AR 72201; and Depts. Physiol. and Biophys., and Psychiat. and The Marine Biomedical Inst., Univ. Texas Med. Branch, Galveston, TX 77550.

The cell bodies of origin of the long descending and ascending propriospinal pathways have been localized in cat and monkey utilizing retrograde transport of the enzyme horseradish peroxidase (HRP). According to other investigators the method of chromatolysis has not been adequate to make this localization.

Injections of 1-5 µl of 50% or 70% HRP in saline were made in either the cervical or lumbar enlargements using a glass pipette of 50-100 µm diameter attached to a microsyringe. Penetrations went through the dorsal columns in order to injure as few axons as possible, and injections were made into the spinal gray matter. After 3 days the animals were perfused with 0.5% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer. The 50 µm sections were treated with standard procedures using diaminobenzidine to obtain a reaction product.

After injection into the lumbar enlargement, neurons containing the reaction product (labeled neurons) were found in the cervical enlargement mainly in laminae VII and VIII with a few cells in V, VI and X in both cat and monkey. The cat also had a few labeled neurons in I and IV.

After cervical injections labeled neurons were located in the lumbar enlargement mainly in laminae VII and VIII with some in I, IV, V, VI, and X in both cat and monkey. The monkey had more labeled cells in lamina I and the cat more in IV. The labeled neurons in lamina IV of the cat could be due to injury to axons of non-primary neurons during penetration through the dorsal columns.

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1630 DORSAL ROOT PROJECTIONS TO THE LUMBAR SPINAL CORD IN THE CAT: A LIGHT AND ELECTRON MICROSCOPIC AUTORADIOGRAPHIC STUDY. R.L. Snyder* (SPON: S.B. Tieman). Dept. Anat., Sch. Med., UCSF, San Francisco, CA. 94143.

The projections of the primary afferents to the spinal cord of cats were examined with autoradiography following injections of up to 0.5 mCi tritiated leucine or lysine into single lumbar dorsal root ganglia. Light microscopy of coronal 1 µ plastic sections at the level of the entry of the labeled dorsal root revealed that the heaviest label was found in the marginal layer (ML) and the substantia gelatinosa (SG) of the dorsal horn regardless of the postoperative interval (3-11 days). More ventral portions of the spinal grey - the nucleus proprius (NP), intermediate grey (IG) and the ventral horn (VH) contained progressively less label. At successive levels rostral and caudal to the root entry, the label within the SG and NP decreased rapidly, while the label in the ML and the rest of the spinal grey decreased more slowly. Thus, two or three segments from the level of the root entry most of the label was found in the ML, IG, and Clarke's column (rostrally). SG at these levels contained little or no label. Lesions of Lissauer's tract (LT) prior to ganglion injection completely abolished the labeling of the ML rostral and caudal to the root entry. At the level of entry the amount of label in the ML was reduced, while the rest of the spinal grey was unaffected. Section of the entering large diameter dorsal root axons along the level of entry of the labeled root midway across the dorsal horn abolished the label in the IG and VH, but left the label in the dorsal horn intact. These results suggest that the axons of LT project primarily, if not exclusively, to the ML, and that some of the dorsal column large diameter axons project into dorsal horn, directly, while others curve around the medial portion of the dorsal horn and then recurve up into NP and SG as classically described.

Electron microscopic autoradiography at the level of the root entry demonstrated that the labeled terminals in the ML, SG, and NP represent morphologically distinct populations with some overlap. In the ML most labeled terminals contained large clear vesicles and numerous large dense cored vesicles. In the SG three terminal types were labeled: terminals similar to those labeled in ML; the "C" terminals (Gobel, 1974), which contained large clear vesicles and a dense matrix; and terminals which contained large clear vesicles and a pale matrix. Only this third terminal type was found labeled in the NP and Clarke's column. Since ML, SG and NP-Clarke's receive projections from different fiber types within the dorsal root with some overlap, these results suggest that each of the fiber types within the dorsal root terminates in a bouton that has a characteristic morphology.

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1631 MULTIUNIT ACTIVITY PATTERNS IN THE BRACHIAL SPINAL CORD OF DYSTROPHIC CHICH EMBRYOS. B.T. Stokes. The Ohio State University, Columbus, Ohio 43210.

The frequency and content of polyneuronal burst discharges were examined in different regions of the cervical spinal cord (segments 14 and 15) of normal and dystrophic chick embryos from days 18 to 20 of incubation. On the dorso-ventral axis in a midlateral position, the pattern of change in burst frequency was quite different in normal and dystrophic embryos. Although bursts were encountered in each in approximately the same region and at about the same frequency, significant declines in frequency were encountered only in deeper regions of the spinal cord of dystrophic embryos. In normal embryos, bursts maintained their frequency independently of the position of the sampling electrode. Qualitative and quantitative analysis of individual burst content in these same regions has revealed that a significant decline in the number of spikes per burst is only found in the dystrophic cord in these same deep regions. Microelectrode marking techniques have revealed that this lower region consists mainly of lamina nine cells of the brachial motor columns. If the frequency and content declines in the dystrophic cord are associated with modifications in the motor outflow from this region, they may have relevance to the neurogenic etiology of muscular dystrophy.

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1632 RESPONSE PROPERTIES OF LATERAL CERVICAL NUCLEUS CELLS IN THE RAT Gideon Urca, Glenn J. Giesler, Jr. and John C. Liebeskind. Dept. Psych., UCLA, Los Angeles, CA 90024.

Recent evidence (Giesler et al., this meeting) has demonstrated the existence of a scattered grouping of cells in the first three segments of the dorsolateral cervical cord. Their location in the dorsal area of the lateral columns, their projection to contralateral thalamus as verified by retrograde transport of HRP, and their morphology indicate that they may be the homologue of the lateral cervical nucleus (LCN) found in cat and monkey. In this study, an attempt was made to characterize the somatosensory inputs and projections of this nucleus by electrophysiological means. Unitary recordings were made in Urethane anesthesized paralyzed rats through micropipettes filled with Pontamine Sky Blue dye. Iontophoretic application of dye was performed at the conclusion of each successful recording tract. Histological examination showed all recorded cells to be within LCN. In the majority of experiments a comb of three bipolar stimulating electrodes was placed at the mesodiencephalic junction to test for thalamic projection using the antidromic activation technique. All cells encountered were tested for their responsiveness to hair movement, pressure, noxious pinch and graded electric shocks to the receptive field.

All cells recorded within the LCN responded to hair movement on at least a portion of the ipsilateral body surface. The vast majority of receptive fields covered more than one body quadrant and often extended to the contralateral side. The largest fields were found to cover the entire body surface including the head. In addition to their responsiveness to hair movement a large proportion of cells was also activated by mild pressure. In contrast to previous reports on the cat LCN, a number of cells were found to respond more vigorously to noxious pinch. Units responding only to nonnoxious stimuli reacted to electrical stimulation of their receptive fields with a low threshold, short duration burst of activity. Response latencies varied between 4 and 15 ms depending on the body region stimulated. In contrast, neurons that also responded to noxious stimuli produced additional late bursts of activity following intense electric shock. In the majority of cases antidromic testing revealed a projection to the contralateral thalamus.

The ability of these cells to code both noxious and nonnoxious stimuli, the remarkable convergence on them, and their direct access to the thalamus all suggest that the LCN has an important role in the perception of cutaneous stimuli in the rat.

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1633 PROTEIN COMPOSITION OF THE SHORT TERM HEMISECTED RAT SPINAL CORD AND BRAIN SHOWN BY POLYACRYLAMIDE SDS SLAB GELS. Michael R. Wells*, Jerald J. Bernstein and Mary E. Bernstein*. Departments of Neuroscience and Opthalmology, University of Florida, Gainesville, Florida, 32610.

Proteins from rat spinal cord and brain were examined using SDS polyacrylamide slab gels at periods of 12 hours, 1, 3, 7 and 14 days after a left spinal cord hemisection at T2. Slab gels (0.75 mm) utilized were essentially the same as those described by Kelly and Luttges (J. Neurochem. 24:1077, 1975) consisting of a linear-exponential gradient from 8.75% to 18.75%. Male Long-Evans hooded rats were used throughout the study. Two hemisected, two sham (laminectomy and dura incisure) and one normal animal were used at each time interval. At the time of utilization animals were perfused with saline. Samples were taken from left and right somatomotor and occipital cortex, and in 4 mm segments from left and right halves of spinal cord extending up to 14 mm rostral and caudal from the site of spinal cord hemisection. Analysis was made by direct comparison of banding patterns on the gel by stain (Coomassie Blue) and densitometry. In brain, no differences in protein patterns or intensity were noted over postoperative time. In spinal cord, banding patterns at the site of lesion indicated increases in the staining intensity of bands in the regions of 10-20,000 molecular weight (MW), 70-75,000 MW, 80-85,000 MW, and 110-120,000 MW as early as 12 hours after hemisection. At sites distal to the lesion, both left and right (bilaterally) only bands in the regions of 70-75,000 MW and 80-85,000 MW showed increases in intensity, primarily the 70-75,000 MW region. This band comprised from 7-10% of the total protein in the area of the lesion. The rostrocaudal extent of the increased band at 70-75,000 MW reached a maximum between 12 hours and one day after hemisection and decreased over time. By 14 days postoperative, the major band was restricted to the site of hemisection and the cord contralateral to the lesion.

The bands of increased density after hemisection could be correlated with blood components. The major band at 70-75,000 MW corresponded to serum albumin, while the band from 10-20,000 MW may be attributed to hemoglobin. Other bands noted seemed associated with blood globulins or fragments. The penetration of blood proteins into spinal cord tissues was attributed to a breakdown of the blood brain barrier. The presence of high concentrations of such exogenous protein in spinal tissues may be associated with traumatic edema. (Supported by grant NS06164, NINCDS).

1634 SUBSTANTIA GELATINOSA UNIT ACTIVITY: NOCICEPTION AND NARCOTICS. George L. Wilcox and David J. Mayer. Dept. of Pharmacology, Univ. of Minn. Med. Sch., Minneapolis, MN 55455 and Dept. of Physiology, Med. College of Va., Richmond, VA 23298.

Presynaptic inhibition of primary afferent sensory fibers in the spinal cord dorsal horn may be an important mechanism of endogenous CNS pain modulation and of narcotic analgesia. The neurons of substantia gelationsa (SG) have been proposed as mediators of presynaptic inhibition in the dorsal horn. We designed experiments to test the hypotheses that SG mediates presynaptic inhibition and controls afferent impulses, and that morphine alters this inhibition. The investigation applied newly developed statistical/electrophysiological techniques to identify SG unit activity in the rat and to systematically study SG responses to noxious electrical stimulation of the footpad. Further studies tested morphine effects on this SG evoked activity.

The experiments were done acutely on chloralose anesthetized, paralyzed rats with the tibial nerve exposed and a laminectomy of lumbar segments two and three. Electrical stimuli were delivered subcutaneously to the plantar surface of the foot, and the tibial nerve compound action potential was monitored to verify Aβ-γ or Aδ stimulation. In some experiments, a $1mm^2$ area near the stimulating dipole was thermally damaged (50°C for 1 min) in an attempt to increase tonic levels of C fiber activity. Presynaptic inhibitory action (primary afferent depolarization, PAD) in the spinal cord was inferred from measurements of the bipolar dorsal root potential (DRP). SG activity was identified on the basis of spatial disparity of amplitude discriminated poststimulus time histograms (PSTH). Action potentials within a low amplitude window (40-100 μV) which occurred at only one of two closely spaced microelectrodes (5 μm tips, 50 μm separation) would most often have originated from neurons small and closely packed relative to the electrode separation. SG neurons uniquely fulfill these anatomical criteria; therefore, unit activity with maximum interelectrode disparity was ascribed to SG neurons. SG neurons so identified showed characteristically long activation (150 ms) after non-noxious stimuli and their peak activity correlated well with the peak of PAD. Stimuli exciting peripheral Aδ fibers and stimuli accompanied by adjacent tissue damage evoked qualitatively different SG responses and PAD time courses. Differences in SG PSTHs were not uniform, however, suggesting SG comprises a heterogeneous population with respect to nociception. Morphine depressed DRPs to nonoxious as well as noxious stimuli, and appeared to alter the temporal coherence of SG responses. (Supported in part by NIH grants, DE00116 and DA00576, and by grants from Univ. of Minn. Grad. Sch. and The Minnesota Medical Foundation.)

1635 METABOLIC AND FUNCTIONAL CHANGES OF SPINAL CORD IN EXPERIMENTAL ISCHEMIA. Shokei Yamada,* Delmar C. Sanders,* Gary E. Haugen,* and Dale E. Brown.* (SPON: M. Rosenthal) Section of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, CA 92354.

Evidence increasingly suggests that human paraplegia is often the result of spinal cord ischemia. The causes of ischemia have been reported as occlusion of the anterior spinal artery, cord compression by a large intraspinal mass, temporary aortic clamping in repair of coarctation (0.5% incidence), etc. In this investigation the authors have adopted a non-invasive optical technique, to determine changes in the oxidative metabolism of cord mitochondria. Other parameters that were measured included oxygen availability by polarographic oximetry, spinal cord potentials in response to dorsal root stimulation.

Twenty cats were anesthetized with ketamine then intubated and given N_2O:O_2 anesthesia. The lumbosacral cord and nerve roots were exposed. Ischemia of the lower spinal cord was produced by either intraluminal occlusion of the thoracic aorta (Swan-Ganz Catheter) or application of a ligature around the proximal descending aorta. In both cases the femoral arterial blood pressure dropped to 0 mmHg on occlusion. A dual wavelength reflectance spectrophotometer was applied to the lumbosacral cord, and the relative reduction-oxidation (redox) level of cytochrome a,a_3 was measured during various durations of ischemia, ranging from 1 to 10 minutes. The polarographic electrode (25μ teflon coated platinum wire) was inserted into the gray matter through the dorsal column. A bipolar stimulator electrode was applied to the L_7 dorsal nerve root, and a monopolar electrode was applied to the dorso-lateral surface of the L_7 cord segment.

Aortic occlusion resulted in reduction of cytochrome a,a_3, decreased spinal cord oxygen availability, a rapid decrease in the interneuron potential within the first one minute and a minimal decrease in the posterior column potential for the same period. Within one minute after the occlusion for 1 to 5 minutes, the redox level of cytochrome a,a_3 and oxygen availability returned to the original level. But it took longer for the redox level to return to normal after prolonged occlusion. Longer the period of ischemia the slower the return of the interneuron potential to normal. There was little recovery of the interneuron potential noted after the ischemia lasted 10 minutes or longer.

The authors conclude that the ischemia of spinal cord results in depletion of ATP due to the lack of oxygen availability as well as complete reduction of the respiratory chain. The potential change in the interneuron indicates that the ion pump is no longer adequate to maintain the membrane potential because cytochrome a,a_3 were fully reduced.

SYNAPTIC TRANSMISSION

1636 RELEASE OF RADIOLABELED NEUROTRANSMITTERS FROM PURIFIED RAT BRAIN SYNAPTIC VESICLES. Suad Al-Zahid*, Larry J. Bearden* and Samuel T. Christian. Neurosciences Program, Med. Ctr., Univ. Ala. in Birmingham, Birmingham, Ala. 35294.

A flow dialysis system was utilized to study the release of radioactive neurotransmitters from preloaded synaptic vesicles in vitro. Loading of the vesicles was accomplished by incubating highly purified preparations of rat cerebral synaptic vesicles (100 ug protein/ml) with the transmitter to be studied for 10 minutes at 37°C in 5mM Tris-HCl buffer, pH 7.5, containing 156mM KCl, 5mM NaCl, 0.1mM ATP, 0.1mM $MgCl_2$ and 0.1mM EGTA. Preliminary results indicate that vesicular uptake of the putative neurotransmitter serotonin (5HT) is an active process which is efficient at an ATP concentration of 10^{-4}M.

The dialysis cell consisted of an upper chamber (with an injection port) separated from a lower chamber by a cellulose membrane with a M. W. cutoff of 12,000. Entrance and exit ports in the lower chamber permitted dialysis buffer (5mM Tris, pH 7.5, 156mM KCl, 5mM NaCl, 0.1mM ATP, 0.1mM $MgCl_2$, 0.1mM EGTA) to be pumped through the cell continuously. Effluent fractions were collected and subsequently analyzed for the presence of radioactivity. Using this system, the release of ^{3}H-5HT from synaptic vesicles was monitored continuously as components of the release mechanism (synaptic vesicles, synaptic membranes, ATP and cations) were injected sequentially into the upper chamber of the dialysis cell. After the injection of preloaded vesicles into the upper chamber, the rate of efflux of ^{3}H-5HT was allowed to reach a steady state. When vesicles were suspended in the Ca^{++} free dialysis buffer, the subsequent introduction of synaptosomal membranes did not bring about an increase in the rate of efflux of the labeled amine. However, when $2x10^{-6}$M Ca^{++} was titrated into the upper chamber, a rapid efflux of the amine was immediately observed. Deletion of ATP from the dialysis medium was found to abolish the Ca^{++} stimulated ^{3}H-5HT release. No increase in the rate of baseline efflux was observed when Ca^{++} was replaced by Mg^{++} at $2x10^{-6}$M. These experiments show that Ca^{++} and an energy source such as ATP must be present for the release of ^{3}H-5HT to occur. The flow dialysis system mimics the in vivo neurotransmitter release process, and has proven itself to be well suited for further studies of the neurotransmitter release mechanism.

1637 REDUCTION OF POST-TETANIC POTENTIATION BY HALOTHANE IN THE STELLATE GANGLION. Daryl Christ* (SPON: T.W. Schoultz). Dept. Pharmacology, University of Arkansas for Medical Sciences, Little Rock, AR 72201

Compound action potentials were recorded from the postganglionic nerve of the isolated hamster stellate ganglion. Repetitive stimulation (30 Hz for 5 sec) of the preganglionic nerve induced a period of post-tetanic potentiation (p.t.p.) when the ganglion was blocked by hexamethonium chloride (5×10^{-4}M). Halothane reduced the magnitude and duration of the p.t.p. in the presence of hexamethonium. It also reduced the amplitude of the pre-train compound action potential. When the ganglion was blocked with low concentrations of halothane (10-15 mg%) alone, the p.t.p. was very prominent, but it was smaller than the p.t.p. in hexamethonium alone. As the concentration of halothane was increased to 20 mg%, the duration of the p.t.p. was shortened. At 30 mg% halothane, the pre-train potential was completely obliterated, as well as the p.t.p. These results indicate that halothane depresses p.t.p. in the stellate ganglion of the hamster, although the concentrations necessary for this effect are rather large. The depression appears to be due to an effect on the underlying mechanisms of the p.t.p. and not simply due to the blocking action of halothane on the nicotinic effects of the neurotransmitter. (Supported by NIH Grant NS-10393.)

1638 SYNEXIN: A NEW PROTEIN FROM THE ADRENAL MEDULLA THAT MEDIATES CALCIUM DEPENDENT ASSOCIATION OF CHROMAFFIN GRANULES. C.E. Creutz*, C.J. Pazoles and H.B. Pollard (SPON: I. Hanbauer). NIH, Bethesda, MD 20014

Exocytosis of neurotransmitters, hormones and other cell secretory products is generally believed to require an increase in the intracellular free [Ca^{++}], although the mechanism of this Ca^{++} action remains unresolved. The main consequence of Ca^{++} action is thought to be the induction of close association or "fusion" of secretory vesicles with plasma membranes or in many cases, with other secretory vesicles. In order to investigate the biochemical details of this event, we have chosen to study the calcium dependent process of vesicle aggregation using chromaffin granules (CG's) from the adrenal medulla. The aggregation of CG's can be conveniently assayed by turbidity measurements of granule suspensions, and can be verified by light and electron microscopy.

We now report the discovery of a new protein from the adrenal medulla that mediates calcium induced aggregation of CG's. This protein is soluble, heat labile and trypsin sensitive, and can be partially purified by fractionation with ammonium sulfate precipitation and gel filtration. The activity of this protein is absolutely dependent upon a free [Ca^{++}] of at least 10^{-6} M, and under these conditions also binds to CG's. On SDS gels, ~70% of the protein with this activity migrates with an apparent M.W. ~44,000. Electron micrographs of CG aggregates formed by this protein reveal that the limiting membranes of the granules have fused with one another, leaving the individual granule cores separated by a pentilaminar membrane structure. These fusion sites are similar in appearance to the fusion sites seen in E.M. images of secreting cells.

We have named the CG aggregating protein "synexin," from the Greek synexis, which means "meeting". It is possible that synexin is the intracellular calcium receptor in exocytosis and that its role is to regulate membrane fusion.

1639 POSSIBLE ROLE OF SYNAPTIC VESICLE - ASSOCIATED PHOSPHOPROTEINS IN MEDIATING CALCIUM - DEPENDENT NEUROTRANSMITTER RELEASE. Robert J. DeLorenzo and Steven D. Freedman.* Dept. Neurology, Yale Univ. Sch. Med., New Haven, Ct. 06510

The anticonvulsant phenytoin (diphenylhydantoin, DPH) has been shown to inhibit several calcium-dependent release processes, including the release of neurotransmitter at the neuromuscular junction (Ann. Neurol. 1: 334, 1977), from brain slices (Arch. Neurol. 29: 239, 1973), and during posttetanic potentiation (Pharmacol. Exp. Ther. 156: 591, 1967). Previous investigations in this laboratory (BBRC 71: 590, 1976; J. Neurochem. 28: 21, 1977) have demonstrated that DPH and calcium have antagonistic actions on the level of phosphorylation of synaptosomal fraction proteins DPH-L and DPH-M, and presented the hypothesis that these antagonistic effects on protein phosphorylation may mediate the opposing actions of DPH and calcium on neurotransmitter release from the presynaptic nerve terminal.

Since synaptosome preparations are very heterogeneous, the current investigation was initiated to further test this hypothesis by determining the localization of phosphoproteins DPH-L and DPH-M in subfrations prepared from synaptosome preparations by standard density gradient techniques. The synaptosomal preparation was subfractionated into synaptic membrane, synaptosomal mitochondria, synaptosomal soluble, and synaptic vesicle enriched fractions. Each presynaptic nerve terminal preparation subfraction was incubated with [γ-^{32}P] ATP in the presence or absence of calcium and/or DPH and the incorporation of [^{32}P] phosphate into protein in each subfraction was quantitated as described in our previous investigations. Synaptosomal phosphoproteins DPH-L and DPH-M were enriched in highly purified synaptic vesicle fractions, while synaptic membrane and mitochondrial fractions had low levels of these phosphoproteins. DPH inhibited the calcium-dependent phosphorylation of these synaptic vesicle-associated phosphoproteins. The effects of DPH and calcium on the levels of phosphorylation of synaptic vesicle-associated proteins DPH-L and DPH-M were independent of ATP concentration over a wide range of concentrations.

The enrichment of proteins DPH-L and DPH-M in synaptic vesicle fractions suggests that these proteins are present within the presynaptic nerve terminal. These results confirm our initial hypothesis and further suggest that the antagonistic actions of calcium and DPH on neurotransmitter release may be mediated by the opposing actions of these agents on the phosphorylation of specific proteins localized in the presynaptic nerve terminal in association with the synaptic vesicles. Further studies on these synaptic vesicle phosphoproteins may help elucidate the molecular mechanism mediating calcium dependent neurotransmitter release.

1640 ENHANCEMENT OF SYNAPTIC RESPONSES TO STIMULATION OF THE PERFORANT PATH IS DEPENDENT ON THE NUMBER OF FIBERS ACTIVATED. R. M. Douglas and B. L. McNaughton (spon. G. V. Goddard), Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

Long-lasting increases in synaptic efficacy (enhancement) have been observed at the perforant path-granule cell synapse of the hippocampus, but the amount of change has been quite variable. The present study shows that stimulus intensity is a critical factor in determining if, and how much, enhancement is produced by high-frequency stimulation. Small monosynaptic extracellular EPSP's were recorded in the dentate gyrus of rats anaesthetized with pentobarbital. The intensity of test stimuli was held constant while the intensity during the high-frequency trains was varied by changing either pulse width or current. A minimum intensity was needed to produce enhancement. The results from 15 preparations indicated that a stimulus intensity resulting in an EPSP (recorded from the region of maximal positive response in the hilus of 5.6 mV $\pm$ 1.3 (S.D.) was necessary before any enhancement was observed. Trains with intensities yielding larger EPSPs produced more enhancement. while the 5.6 mV EPSPs were close to population spike threshold, the generation of a large population spike by inserting one high-intensity pulse during a low-intensity train did not produce enhancement. Several observations suggest that this effect was due to the activation of more fibers by the high-intensity trains and not to more reliable firing of fibers. Fiber potentials followed equally well during low- and high-intensity trains. More importantly, the low-intensity trains were as effective as the high-intensity trains for producing the short-lasting augmentation-like and PTP-like increases described by McNaughton (this meeting). Furthermore, very long trains of low intensity produced no long-lasting effects, while high-intensity trains of far fewer pulses at much lower frequencies did. The data suggests that the amount of enhancement is proportional to the number of fibers (above an apparent minimum) activated by the high-frequency trains.

1641 THE MEMBRANE EFFECT OF DIBUTYRYL-GUANOSINE 3':5'-CYCLIC MONOPHOSPHATE ON SINGLE MAMMALIAN SYMPATHETIC NEURONS. N.J. Dun, K. Kaibara* and A.G. Karczmar. Loyola Univ. Stritch Sch. Med., Maywood, Il. 60153.

Acetylcholine (ACh) and dibutyryl-guanosine 3':5'-cyclic monophosphate (cGMP) were applied iontophoretically from double barreled micropipettes to the isolated rabbit superior cervical ganglion cells and the responses were recorded by means of intracellular microelectrode techniques. The proximity of the double barraled micropipette was estimated by the rise time of ACh potential (<30 msec) which was induced by a brief single current pulse. cGMP when applied by tetanic current pulses (30 Hz, 5 sec) consistently evoked a membrane depolarization of less than 10 mV in amplitude and 30-120 sec in duration. In many cells, a long after-hyperpolarization was observed following the membrane depolarization elicited by cGMP. The cGMP induced membrane depolarization was not blocked by superfusing the ganglion with d-tubocurarine (50 µM) which completely abolished the ACh potential and/or atropine (10 µM). Replacing Na ions from the superfusing solution with Tris-buffer which completely and reversibly abolished the ACh potential did not appreciably affect the cGMP induced membrane depolarization. Other ionic studies revealed that the electrogenesis of cGMP mediated membrane depolarization was complex and apparently different from the usual ACh potential generated by nicotinic receptors. (Supported by NS06455).

1642 ACTIVATION OF FROG MOTONEURONS BY STIMULATION OF CONTRALATERAL VENTRAL ROOTS. S. D. Erulkar and R. W. Soller*. Dept. Pharmacology, Univ. Penna. Sch. Med., Philadelphia, Pa. 19174.

It has been shown by electrophysiological techniques that lumbar motoneurons on the same side of the frog spinal cord are electrically coupled (Grinnell, J. Physiol. 182: 612, 1966). This coupling is thought to occur through motoneuronal dendrites which ramify within the dorsal horn. Using the Golgi method Liu and Chambers (The Interneuron, U. Calif. Press: 193, 1969) have shown the presence of medially oriented motoneuronal dendrites which cross the midline in the anterior commissure. In this study we demonstrate electrical interaction among motoneurons of both sides of segments 8 - 10 of the isolated frog (R. pipiens) spinal cord and suggest that this may occur through bundling of the medially directed dendrites.

Electrical stimulation of ventral roots 8, 9 or 10 on one side elicited responses in respective contralateral ventral roots; these responses were characterized by two components: a short latency (1-4 msec.) spike-like response followed by asynchronous activity which was sustained for 50 msec. The second component was abolished by substituting the normal Ringer's solution with one containing no calcium and 2 mM magnesium, suggesting that the asynchronous activity was due to synaptic activation, while the early response resulted from electrical interaction. It was unclear whether the synaptically evoked activity was a result of crossed recurrent pathways activated by motoneuronal collaterals or pathways activated secondarily to electrical stimulation.

Intracellular recordings from motoneurons in normal Ringer's solution confirmed the presence of both components of motoneuronal activation in response to contralateral ventral root stimulation. The responses with the shortest latencies were action potentials whose ascending phases rose directly from the baseline; in later responses, the action potentials were generated from EPSP's. In zero calcium Ringer's solution only short latency spikes were elicited. Furthermore, in many units the latencies of contralaterally evoked spikes were shorter than those of ipsilaterally evoked spikes.

Both electrical and synaptic interactions among motoneurons were transmitted through the anterior commissure, since cutting this structure abolished the cross-segmental interaction.

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1643 EFFECTS OF POSTSYNAPTIC COBALT INJECTIONS ON VERTEBRATE ELECTRICAL AND CHEMICAL SYNAPTIC TRANSMISSION. Donald S. Faber, Charles Kaars*, and Steven J. Zottoli. Res. Inst. on Alcoholism, Buffalo, N.Y. 14203 and Depts. of Biology and Physiology, SUNYAB.

Iontophoretic injections of cobalt ions into the goldfish Mauthner cell (M-cell) have marked effects on mixed electrically and chemically mediated excitatory postsynaptic potentials (EPSPs) evoked by stimulation of the posterior branch of the VIIIth nerve. Intracellular recordings were obtained from the M-cell soma or lateral dendrite with cobalt-acetate microelectrodes and a second independently positioned KCl electrode. Using 2.5-50nA current pulses of 100-200 msec duration at intervals of 1.5 sec we have always observed an irreversible reduction or blockade of the electrotonic component of the mixed PSP. This decoupling occurs in the absence of any significant reduction in M-cell resting membrane potential, antidromic spike height, or input resistance. Cobalt also brings about a reversible reduction (20-50%) in the later chemical component of the mixed PSP. Selective stimulation of VIIIth nerve branches can produce chemically mediated EPSPs in the M-cell which are not associated with a shorter latency electrotonic PSP. In contrast to the observed reduction of the chemical component of the mixed responses, these exclusively chemical PSPs are not reduced by cobalt. Histological localization of the injected Co^{++} confirmed that it was restricted to the M-cell.

Politoff et al., (Brain Res. 76:343, 1974) found that Co^{++} crossed the electrotonic synapses connecting adjacent segments of the crayfish lateral giant axon when iontophoresed slowly and decoupled these synapses when iontophoresed rapidly. Our results indicate that intracellular Co^{++} can also decouple a vertebrate electrotonic synapse. The lack of an effect on the passive membrane properties of the M-cell indicates that Co^{++} - induced decoupling results from an increased junctional resistance. Nakajima (J. Comp. Neur. 156:375, 1974) has found that the presynaptic terminals electrotonically coupled to the M-cell lateral dendrite are morphologically mixed, i.e. they exhibit both gap junctions and the ultrastructural specializations commonly associated with chemical transmission. The observation that Co^{++} can reduce the chemical component of mixed PSPs while having no effect on purely chemically mediated PSPs suggests that Co^{++} crosses these gap junctions and exerts a presynaptic effect. The results are consistent with the hypothesis that synapses with the morphological correlates of both electrical and chemical transmission ordinarily function in both of these modes.
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1644 INTRAVENTRICULAR ADMINISTRATION OF POTENT EXCITOTOXINS MAY YIELD CLUES TO LOCATION OF GLUTARECEPTIVE NEURONS. T. de Gubareff* and J.W. Olney, Washington Univ. Sch. Med., St. Louis, MO 63110.

Kainate (KA), N-methyl-DL-aspartate (NMA) and DL-homocysteate (HCA) are potent excitatory analogs of glutamate (GLU) which, when administered systemically, mimic GLU in destroying neurons in or near circumventricular organ (CVO) regions of brain (e.g., arcuate hypothalamic nucleus, subfornical organ, area postrema). They also destroy neurons locally when injected directly into various brain regions. The toxic action of these compounds appears to be selective for dendritic and somal, while sparing axonal portions of the neuron (Olney, et al., NS Abst., 1, 371, '75); however, it remains to be clarified whether or to what extent the toxic action is specific for functionally distinct populations of neurons. To explore this, we administered these agents into the lateral ventricle of adult rats in doses of 20 nmoles (KA), 150 nmoles (NMA) and 600 nmoles (HCA) and comprehensively examined the CNS for acute lesions. The spinal cord and brain stem were included since GLU is suspected of being an excitatory transmitter released at primary afferent sensory synapses (e.g., on spinal dorsal horn interneurons and neurons of gracile, cuneate and spinal trigeminal nuclei.

All KA-treated animals died within 1 hr after treatment; however, a widespread GLU-type dendritotoxic reaction was evident at the time of death which corresponded roughly in distribution with more circumscribed neuron-necrotizing lesions detected in NMA or HCA animals three hours following treatment. Major findings were that neurons in CVO regions tended to be spared while those in several non-CVO regions were selectively damaged. Neural elements upon which primary sensory afferent fibers synapse were particularly vulnerable as were stellate interneurons in the molecular layer of the cerebellar cortex and certain hippocampal interneurons.

We conclude: (1) that systemic administration of excitatory amino acids may be preferable to intraventricular administration for purposes of exploring the role(s) of arcuate or other CVO neurons in neuroendocrine function since excitatory amino acids may have greater access to CVO zones from blood than from the ventricle. (2) Excitatory amino acids given intraventricularly may be preferentially toxic to neural elements having glutamergic (or aspartergic?) synaptic input, although non-uniform and incomplete penetration of these compounds from ventricle into brain precludes obtaining more than a partial mapping of such elements. (3) Whether glutareceptive and aspareceptive elements can be identified separately by intraventricular application of potent analogs related structurally more to one or the other of these putative transmitters is an intriguing issue to be explored. Supported by USPH grants NS-09156, DA-00259 and RSD Award MH-38894 (JWO).

1645 FACILITATION OF CALCIUM-ACTIVATED OUTWARD CURRENT IN SKATE AMPULLAE OF LORENZINI. W.D. Huse*, D.C. Spray and M.V.L. Bennett, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Voltage-clamped ampullae of Lorenzini exhibit two phases of active current generated by the receptor cells when stimulated by an appropriate command pulse. An early inward phase is carried by Ca, voltage-sensitive, and non-inactivating. A late outward phase (I_{out}) is voltage-insensitive, dependent on Ca influx, and presumably carried by potassium ions (cf. Clusin, Spray & Bennett, Nature, 256: 425, 1975; Clusin & Bennett, J. Gen. Physiol., 69: 145, 1977). I_{out} tail currents last about 0.6 sec, which may reflect time for reduction of intracellular free Ca. For several seconds after a Ca influx, activation of I_{out} requires less Ca influx, which suggests that residual Ca below the level required for I_{out} is removed gradually over this period. For pulses briefer than 0.2 sec a constant Ca influx is required for outward current activation (I_{out}), i.e. Ca removal is insignificant over this period, and brief pulses threshold for I_{out} can be used as a quantitative measure of residual Ca. Ca influxes subthreshold for I_{out} can also be quantitatively measured, but when I_{out} is activated the cells escape from the clamp and influx is no longer easily determined. For increasing pulses of constant duration that maximally activate g_{Ca}, but are too brief to activate I_{out}, facilitation decreases as the Ca equilibrium potential E_{Ca} is approached, i.e. less influx produces less facilitation. For a pulse exceeding the E_{Ca} there is still some facilitation ascribable to a Ca tail current after the pulse; this current is difficult to see directly because of capacitative transients. Below E_{Ca} longer pulses produce greater facilitation. Above E_{Ca} increasing pulse duration does not affect facilitation, which is consistent with the tail current explanation. Apparently the time course of facilitation is longer following prepulses that themselves activate the late current; the calcium influx under these conditions is much larger and removal mechanisms may be altered. Analysis of the time course of facilitation following Ca influxes of varying amounts should help to clarify the nature of Ca removal mechanisms in the receptor cells.

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1646 PRESYNAPTIC RECEPTORS MAY REGULATE ACETYLCHOLINE RELEASE IN PARASYMPATHETIC GANGLIA. David A. Johnson*, Robert Beach*, Jesus Alanis and Guillermo Pilar. Physiology Section, Biological Sciences Group, University of Connecticut, Storrs, CT 06268.

In adrenergic systems, transmitter release is influenced by presynaptic receptors. Similar modulation has been suggested to exist in cholinergic systems (Polak, R.L., Br. J. Pharmac. 41: 600, 1971); however, the mechanism of action has not been shown. The present study demonstrates the presence of presynaptic ACh receptors in the avian ciliary ganglion and characterizes their role in the release of neurotransmitter.

Ganglia from 5-9 day-old White Leghorn chicks were prelabelled with 1μM ^{3}H-Ch at 37°C. ^{3}H-Ch taken up from the incubation medium was synthesized into ^{3}H-ACh. ^{3}H-Ch and ^{3}H-ACh were separated by liquid ion exchange following the conversion of ^{3}H-Ch to ^{3}H-PhCh by choline kinase. Tetraphenyl boron in acetonitrile: toluene fluor extracts ^{3}H-ACh (>85%) while the resultant ^{3}H-PhCh remains in the aqueous phase (>98%). Labelled ACh remaining in the ganglion at the conclusion of the experiment was determined by high voltage electrophoresis following extraction with acetic acid:ethanol.

In the presence of 35μM neostismine bromide, effluents were collected at 3 min intervals. ^{3}H-ACh was released by 3 min. 50 mM K^+ challenges or 3 min. preganglionic nerve stimulation at 20 Hz. This release was shown to be Ca^{++}-dependent. The ^{3}H-ACh released by electrical stimulation was less than that brought about by high K^+. More than 60% of all radioactivity collected during stimulation was identified as ^{3}H-ACh. Atropine (3.0×10^{-6} M), added to the bath, caused a significant increase in the level of ^{3}H-ACh released in response to stimulation by high K^+.

Transient ACh application (3.2×10^{-7} M), while recording intracellularly from presynaptic nerve terminals, elicited a 5-10 mV reduction in membrane potential accompanied by a decrease in membrane conductance. The presynaptic action potential amplitude was also reduced. Prolonged bath ACh application results in a decreased excitatory post-synaptic potential which is partially due to post-synaptic receptor desensitization. There was a decreased quantal content, measured by the coefficient of variation during this period, suggestive of a decrease in transmitter release.

This evidence supports the hypothesis that there are ACh receptors in preganglionic nerve terminals and they may be involved in modulation of transmitter release through a feedback mechanism via presynaptic depolarization.

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1647 EFFECTS OF CADMIUM ON CALCIUM-DEPENDENT SYNAPTIC TRANSMISSION IN THE BULLFROG SYMPATHETIC GANGLION. T.E. Kober* and G.P. Cooper. Dept. Environ. Health, Col. Med., U. Cincinnati, Cincinnati, OH 45267.

In recent work (Nature 262: 704, 1976) with bullfrog sympathetic ganglia, we showed, using electrophysiological techniques, that lead blocks synaptic transmission presynaptically by competition with calcium and, using ^{45}Ca, that lead in fact does reduce the uptake of calcium by preganglionic nerve terminals. These experiments have now been replicated with cadmium.

The 9th or 10th sympathetic ganglion was removed from the bullfrog (Rana catesbeiana) along with several cm of the pre- and postganglionic nerves. The ganglion was placed in a chamber which allowed for the perfusion of the ganglion with normal or modified Ringer's solution. Supramaximal stimuli were applied at a rate of 1/sec. Control response was measured after 30 min in normal Ringer. Then the ganglion was perfused for 30 min with Ringer's solution containing lowered Ca, added Cd ($CdCl_2$), or both. The log of the ganglionic response amplitude was a positive linear function of the log of Ca concentration between 0.2 and 1.0 µM Ca. Cd shifted the response to the right with no appreciable change in slope, suggesting a competitive antagonism of Ca by Cd.

In other experiments the effects of Cd on the uptake of ^{45}Ca by presynaptic nerve terminals were examined. The method of Blaustein was used (Science 172: 391, 1971). ^{45}Ca was added to a bath containing a pair of ganglia. One member of the pair was stimulated supramaximally for 20 minutes at a rate of 6 stimuli/sec. The amount of ^{45}Ca taken into the ganglia was measured by conventional scintillation counting. Stimulation of the preganglionic nerve in control preparations resulted in a three-fold increase in ^{45}Ca uptake. However, 50µM Ca reduced ^{45}Ca uptake of stimulated ganglia by only 30% (the same concentration of Pb reduced the uptake by 91%).

The electrophysiological experiments suggest that Cd, like Pb, blocks synaptic transmission in the sympathetic ganglion by competitive antagonism of Ca-mediated transmitter release from presynaptic nerve terminals. However, Cd has appreciably less effect on the entry of ^{45}Ca into presynaptic nerve terminals. It is possible that Cd and Pb interfere with Ca at different cellular loci. (Supported by EPA contract #68-03-0429 and NIEHS grants #ES-00159 and #ES-01494).

1648 POSSIBLE ROLE OF cAMP IN MODULATION OF ENDOGENOUS ACTIVITY IN THE LOBSTER CARDIAC GANGLION. José R. Lemos* and Allan Berlind. Biology Department, Wesleyan University, Middletown, CT. 06457.

In the lobster, Homarus americanus, the pericardial organ releases a putative hormone which acts upon the heart. The hormone has been identified as a peptide (Berlind and Cooke, 1970) and the pericardial organ extract (XPO) when applied to the isolated cardiac ganglion increases both the frequency of bursts and the number of spikes (action potentials) within each burst.

5-hydroxytryptamine (5-HT), a putative neurotransmitter in the ganglion, increased the frequency of endogenous bursting but appeared to have no effect on the number of spikes per burst.

Both cAMP and its analogs, $N^6,O^{2'}$-dibutyryl cAMP and 8'-Bromo cAMP, mimicked the action of XPO when applied to the isolated ganglion. Dibutyryl cAMP produced significant increases in concentrations as low as 10^{-8}M. The effects of cAMP were linearly related to its applied concentration. Sodium butyrate (10^{-5}M) and 5' AMP (10^{-5}) decreased bursting frequency. Cinanserin (5×10^{-5}M), a 5HT antagonist, inhibited the endogenous activity of the ganglion.

A number of cyclic nucleotide phosphodiesterase inhibitors (PI): isobutyl methyl xanthine (10^{-5}), aminophylline (10^{-5}) and papaverine (10^{-4}) mimicked the effects of XPO. Preliminary results indicate that these PI elevate the levels of cAMP in the ganglion 4 to 5-fold. Furthermore, the PI's potentiate the effects of XPO. Evidence will be presented on the direct effect of XPO application on cAMP levels in the 9-neuron system and the localization of cAMP within that system.

The system affords an opportunity to determine the mechanism for modulation of activity in a small and relatively simple neuronal network.

1649 FUNCTIONAL AND ANATOMICAL STUDY OF SYNAPTIC TRANSMISSION IN CULTURE WITH PRESYNAPTIC HORSERADISH PEROXIDASE INJECTION. R.L. Macdonald, E.A. Neale and P.G. Nelson. BBBr., NICHD, NIH, Bethesda, Md. 20014.

Mammalian neurons derived from fetal mouse spinal cord and grown in dissociated cell culture have been used to investigate the relationship between morphology and function in central nervous system synapses. Evoked monosynaptic postsynaptic potentials (PSP's) were recorded during simultaneous intracellular recordings from pairs of synaptically connected neurons; after electrophysiological study, the presynaptic neurons were injected with horseradish peroxidase (HRP) by passing 0.5 sec positive pulses for 40-100 nA.min at 1/sec through the recording micropipette which was filled with a 4% solution of HRP (Sigma, Type VI) in Tris-HCl and 0.2 M KCl (pH 8.6). Following fixation and incubation for peroxidase activity, it was possible at the light and electron microscopic (EM) levels to determine the number and distribution of presynaptic terminals, thus allowing a direct comparison between individual PSPs and their corresponding terminals. Evoked monosynaptic inhibitory postsynaptic potentials (IPSPs) were observed less frequently than evoked monosynaptic excitatory postsynaptic potentials (EPSPs). IPSPs were monophasic with rapid rise and fall time and were easily reversed from hyperpolarizing to depolarizing by passing polarizing current through the recording microelectrode with reversal potentials being 10-20 mV hyperpolarized relative to the resting membrane potential. HRP injection revealed that although the somas of inhibitory neurons were usually small in size, the dendrites often manifested a complex morphology with numerous highly branched, entwined processes. These neurons preferentially distributed their synaptic terminals to somas and proximal dendrites of postsynaptic cells. EPSPs were variable in size (1-40 mV) and in time course. The excitatory neurons generally had large somas from which emerged several moderately branched, tapering dendrites. In one excitatory example, the EPSP was 3-4 mV and HRP injection revealed 28 synaptic terminals distributed along the soma and only 2 of the postsynaptic cell dendrites. EM reconstruction of 10 boutons revealed an average of 3-4 areas of synaptic specialization (presumed release sites) to yield a total number of 75-100 release sites. Assumption of a quantal size of 100-200 μV (based on previous experiments using coefficient of variation measurements) implies a quantal content of 15-40 and suggests a quantal content per terminal of about 1, with a release probability per release site of 0.2 to 0.3. The results suggest that in dissociated cell culture, spinal cord neurons retain significant specificity with respect to function and morphology.

1650 CHARACTERIZATION OF BRAIN STEM NEURONS IN CULTURES. Kenneth C. Marshall, Walter J. Hendelman and Boris Gimbarzevsky. Depts. of Physiology and Anatomy, University of Ottawa, Ottawa, Canada, K1N 9A9.

Cultures of cerebellum explanted from newborn mice into the Maximow chamber mature in an organotypic manner. Explants from the peduncular region typically contain three distinct groupings of neurons, a cortex region (Cx), one containing deep nuclear neurons (DN), and an area of brain stem neurons (BS). The identity of these BS cells has not been ascertained, but we have characterized them in morphological and electrophysiological studies in the whole explants described above, and in cultures which consisted exclusively of BS neurons. In living cultures, it is seen that the typical BS neurons are 20-25 μ diameter, often with granules around the periphery of the soma and are found in a compact cluster. In studies of such neurons injected with horseradish peroxidase, they are found to have stout, and very long dendrites bearing spine-like processes. The axons branch extensively and course to widespread regions of the cultures. Electrophysiological studies in which two BS neurons are recorded simultaneously by different electrodes have shown that excitatory synaptic connections are formed between these cells. Cross-correlation analysis of extracellular records demonstrated short-latency excitatory correlations. Intracellular records reveal EPSPs associated with iontophoretic activation of a second BS neuron. Electrical stimulation of the cortical region in these cultures may result in short latency EPSPs or antidromic activation. The EPSPs are thought to be the result of collateral activation from other antidromically excited BS neurons. Electron microscopic studies indicate an absence of synapses on the soma and proximal dendrites, but asymmetric synapses are formed on small post synaptic profiles by boutons containing round vesicles. These are also seen in cultures containing peduncular region alone, indicating the origin of the boutons from BS neurons. These studies will aid in the identification of the BS neurons, and may lead to a model system for study of a CNS excitatory transmitter.

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1651 PHYSIOLOGICAL BASIS OF DEPOLARIZING AND HYPERPOLARIZING EVOKED POTENTIALS IN GIANT AXONS IN LAMPREY SPINAL CORD. Gary Matthews* and Warren O. Wickelgren. Dept. Physiol., Univ. of Colo. Med. Sch., Denver, CO 80262.

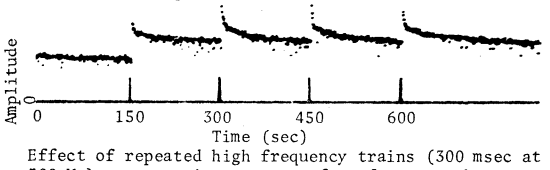
Intracellular recordings were made from the spinal axons of reticulospinal cells (Müller cells) in lamprey. Depolarizing and hyperpolarizing "synaptic-like" potentials were observed in response to stimulation of the spinal cord several cm distant. Their amplitudes were graded with the intensity of the stimulus to the spinal cord but never were larger than 10 mV at normal resting potential (-80 mV). The amplitude of the hyperpolarizing response was not affected by hyperpolarization with injected current, and no change in input resistance during the response could be detected. Therefore, it is proposed that the hyperpolarizing response is caused by the passive spread of inhibitory postsynaptic potentials from cells electrically coupled to Müller axons. The depolarizing response, on the other hand, grew in amplitude as an axon was hyperpolarized to -90 mV, but further hyperpolarization produced no additional increase in response size. Depolarization to -70 mV (which was the limit due to delayed rectification in the axon membrane and the limited current passing capabilities of the electrodes) nearly abolished the response, and the extrapolated "reversal potential" was -65 mV. The dependence of the size of the depolarizing response on axonal membrane potential suggested that it was caused by a conductance change, but the failure of the response to continue to grow with hyperpolarization beyond -90 mV was inconsistent with this interpretation. In fact, if it is recognized that the depolarizing response normally turns on some delayed rectification and thus "shorts" itself out to some extent, then the growth of the response during imposed hyperpolarization of the axon could be due to progressive reduction in the amount of delayed rectification produced by the depolarizing response. Further, it would be expected that the effect of hyperpolarization should saturate when the entire area of axon membrane at which the recording electrode looks has been hyperpolarized sufficiently to prevent the depolarizing response from turning on any delayed rectification. Quantitative analysis of the potential dependence of the response amplitude and the current-voltage characteristics of Müller axons was consistent with this interpretation. Thus, the depolarizing response in Müller axons is apparently also a result of passive spread to them of current from cells to which they are electrically coupled. This work suggests that depolarizing coupling potentials can show dependence on membrane potential and thus can mimic to some extent chemical synaptic potentials. (Supported by NIH grant NS 09661).

1652 DISSOCIATION OF SHORT- AND LONG-LASTING MODIFICATION OF SYNAPTIC EFFICACY AT THE TERMINALS OF THE PERFORANT PATH. B. L. McNaughton. Dept. of Psych., Dalhousie Univ., Halifax, N. S., Canada, B3H 4J1.

When brief trains of high-frequency electrical stimulation are delivered to the perforant pathway, the synaptic response recorded in the fascia dentata undergoes a prolonged increase which can last for weeks in chronically prepared animals. In anaesthetized preparations, the mechanism generating the long-lasting increase was observed to saturate with repeated stimulus trains. After saturation, however, short-lasting increases could be elicited repeatedly. The decay of these short-lasting effects is well fit by a sum of two exponentials whose time constants are similar to those reported by Magelby and Zengle (J.P. 257:449, 1976) for augmentation and post-tetanic potentiation at the neuromuscular junction. This suggests that the long-lasting effect is not PTP but some other process not found at the neuromuscular junction. In the absence of evidence to the contrary, the descriptive term enhancement will be used for the long-lasting process.

A stimulus intensity threshold exists below which only short-term effects but not enhancement can be elicited, regardless of train frequency or duration. It is thus possible to study the short-term effects in the absence of enhancement, during its development, and once the enhancement mechanism has been saturated. These studies indicate that enhancement multiplies the synaptic response by a constant which is independent of the presence or absence of the short-term processes. This is a necessary condition for the localization of the enhancement process to the post-synaptic cell. These studies also provide evidence that enhancement develops slowly, over a period of about 50 seconds following a brief high-frequency stimulus train.

A process resembling facilitation at the neuromuscular junction also occurs at these synapses. This process, which is observed when paired stimuli are delivered at intervals of between 20 to 50 msec, is markedly affected by the presence of the other short-term processes, but not by enhancement.



Effect of repeated high frequency trains (300 msec at 500 Hz) on synaptic response of perforant path. Test pulses at .5 Hz. Trains indicated by vertical bars.

1653 ACTION OF BACLOFEN ON CUNEATE TRANSMISSION. M. E. Morris, K. Krnjević, and S. Fox*. Department of Anaesthesia Research, McGill University, Montreal, Canada.

The antispastic agent Baclofen (*Lioresal*, β-(4-chlorophenyl)-γ-aminobutyric acid) has previously been shown to markedly depress the efficiency of transmission at the primary afferent synapses of the cuneate nucleus, while decreasing the excitability of the presynaptic terminals (Fox et al, 1976, Neurosci. Abs. I, 1003). In further studies multi-barrelled micro-pipettes have been used to record the extracellular activity of single cuneate neurones in unanaesthetized, decerebrate cats and for iontophoretic applications of Baclofen and the potential excitatory transmitters, glutamic acid and Substance P (sP). Outer barrels contained 1M Na-L-glutamate, 10mM sP (Beckman) in 20mM acetic acid, 1M NaCl, and 10-15mM dl-Baclofen (Ciba); the central recording barrel was filled with 3M NaCl. Iontophoretic application of Baclofen blocked rapidly but reversibly all forms of unit firing (spontaneous, or evoked by glutamate or sP) - probably by activating post-synaptic GABA receptors (Puil et al, 1975, Fed. Proc. 35, 307). In contrast, intravenous injections of Baclofen (0.1-10 mg/kg) had no consistent effects on such unit discharges or other excitatory effects of sP, and also did not depress the electrical excitability of the post-synaptic cuneo-thalamic cells (reflected in the α-wave of the medial lemniscal response to direct cuneate stimulation). At the same time afferent terminals became less excitable, as shown by a reduction in (i) the direct antidromic responses, (ii) the dorsal column reflex, and (iii) the enhancement normally evoked by a preceding afferent volley (although the associated inhibition of transmission was not diminished). From these results it appears that the block of transmission by Baclofen is unlikely to be mainly due to a specific antagonism of sP. The most significant site of action is probably presynaptic - either through block of impulse invasion into terminals, or interference with the storage and/or release of transmitter. Since post-tetanic potentiation is maintained or even enhanced, Baclofen cannot cause total depletion of transmitter stores. As previously postulated (Fox et al, 1976, Neurosci. Abs. I, 1003), the most probable mechanism of action is by interference with transmitter release (cf. also recent demonstration of evoked release of amino acids from cortical slices (Potashner, 1976, Fed. Proc. 36, 952)).

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1654 A SPECIFIC EFFECT OF LOW DOSES OF SEROTONIN ON THE DECAY RATE OF POST TETANIC POTENTIATION. Susan A. Newlin*, Paul B. J. Woodson, Werner T. Schlapfer, and Samuel H. Barondes. Div. of Physiology and Pharmacology, Dept. of Medicine, and Dept. of Psychiatry, UCSD, La Jolla, CA 92093; and Psychiatry Research, VAH, San Diego, CA 92161.

Low doses of serotonin (10^{-8} M to 10^{-7} M) in the perfusion media cause an increase in the rate constant of decay (k) of post tetanic potentiation (PTP) at an identified synapse of Aplysia californica, without causing a change in the amplitude of PTP or in the size of an isolated EPSP. With higher doses of serotonin (10^{-5} M) effects on the amplitudes of PTP and on isolated EPSPs are also observed (Tremblay, et al., Brain Res. 109: 61, 1976).

The increase in k caused by serotonin (10^{-7} M) can be strongly antagonized by the serotonin antagonist, SQ 10,631 (10^{-4} M). Serotonin also causes a hyperpolarization of R15 at the hyperpolarized membrane potential we use. This post-synaptic effect is not blocked by SQ 10,631, but is blocked by LSD-25, BOL-148, and Methysergide.

Isobutlymethylxanthine (IBMX)(5×10^{-4} M) + serotonin(10^{-7} M) cause a greater increase in k than either agent alone. IBMX has been shown to inhibit phosphodiesterase in Aplysia (Treistman and Levitan, Nature 261: 62, 1976); and serotonin has been shown to increase cyclic AMP (Cedar and Schwartz, J. Gen. Physiol. 60: 570, 1972; Levitan, et al., J. Neurobiol. 5: 511, 1974) and activate a protein kinase in Aplysia (Levitan and Barondes, PNAS 71: 1145, 1974).

The low effective doses of serotonin and the antagonism of the serotonin effect by SQ 10,631 are consistent with a serotonin effect at a presynaptic receptor at RC1-R15. The effect of IBMX suggests that, as with other known actions of serotonin, a cyclic nucleotide may be involved. The data also suggest that the presynaptic receptor has different pharmacological properties than the serotonin receptors on R15.

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1655 BIOLOGICAL PROPERTIES OF NON-NEUROTOXIC β-BUNGAROTOXIN. Ronald H. Ng* and Bruce D. Howard. (SPON: F.D. Marshall, Jr.) Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.

β-Bungarotoxin is a presynaptically acting neurotoxin that inhibits the evoked release of acetylcholine from motor nerve terminals. The toxin also alters the storage of several transmitters and non-transmitter compounds in synaptosomes. Wernicke et al.[1] have proposed and given evidence that the effects of β-bungarotoxin on neuromuscular and brain synapses result from an interference with energy metabolism caused by a phospholipase A activity associated with the toxin. To investigate the enigma that β-bungarotoxin is neurotoxic while most other phospholipases are not, we examined whether neuronal membranes possess a specific phosphoglyceride substrate for β-bungarotoxin phospholipase A. If found, it may explain the following observations: (1) neuronal membranes are a better substrate than erythrocyte ghosts for β-bungarotoxin phospholipase A[1], and (2) brain membrane preparations contain a higher density of high affinity binding sites for the toxin than do membranes from liver or erythrocytes[2]. However, we found that the extent of different phospholipids hydrolyzed and the composition of fatty acids released from rat brain synaptosomes by β-bungarotoxin did not differ significantly from those affected by a non-neurotoxic phospholipase A from the same snake venom or that from V. russelli venom. β-Bungarotoxin appears to have 2 sites essential for its neurotoxicity: its phospholipase A catalytic site and a second site[3]. By chemically modifying this second site β-bungarotoxin can be converted into a non-neurotoxic phospholipase A. This conversion causes no change in its phospholipase A activity using synaptosomes as substrate. However, non-neurotoxic β-bungarotoxin has lost the native toxin's ability to inhibit GABA uptake into synaptosomes; the inhibition of GABA uptake is due to a toxin-induced depletion of energy stores[1]. These results suggest that the neurotoxicity of β-bungarotoxin cannot be attributed solely to its ability to hydrolyze phosphoglycerides. (Supported by NIH grant NS12873 and NIH postdoctoral fellowship NS05706)

1. J. Neurochem. 25 (1975) 483-496.
2. Biochim. Biophys. Acta 433 (1976) 662-673.
3. Biochemistry 16 (1977) 122-125.

1656 D AND L ISOMERS OF HOMOCYSTEIC ACID COMPARED FOR NEUROTOXICITY. J.W. Olney, T. de Gubareff,* and C.H. Misra. Washington Univ. Sch. Med., St. Louis, MO 63110.

In early microelectrophoretic experiments, Curtis and Watkins demonstrated that L-homocysteic acid (L-HCA) is about equal to glutamate (GLU) in neuroexcitatory potency, whereas D-HCA is substantially more powerful. More recent findings of Balcar and Johnston (J. Neurobiol., 3, 295, 1972) and Cox and Watkins (Brit. J. Pharm., 57, 433, 1976) suggest that L-HCA is taken up by both high and low affinity transport into brain slices whereas D-HCA is not taken up at all. GLU has neurotoxic properties which its several excitatory analogs, including DL-HCA mimic (Olney et al., Exp. Br. Res. 14, 61, 1971). Since rapid intracellular uptake by high affinity transport is the postulated mechanism by which the excitatory stimulus of GLU and its analogs is terminated, failure of D-HCA to be taken up (hence failure to be inactivated) may help explain its greater excitatory potency. Furthermore, if the excitotoxic hypothesis (Olney et al., NS Abst. 1, 371, 1975) is correct, that the excitatory and toxic activities of GLU-mimetic compounds are mediated by a common mechanism acting at a common receptor, it follows that D-HCA, being a more potent excitant than L-HCA, should also be a more potent toxin. If, on the other hand, the toxic activity of GLU-mimetics stems from an interference in intracellular metabolism as some have postulated, only the HCA isomer which is taken up intracellularly (L-HCA) should have appreciable toxic activity.

D-HCA and L-HCA were administered subcutaneously to 10 day-old mice at several doses and their relative neurotoxic potencies were assessed in terms of the severity of the hypothalamic lesion produced at a given dose. Each compound was also injected directly into the striatum of the adult rat and the size of the acute lesion induced by a given dose of each isomer was compared. By either subcutaneous or intrastriatal injection both isomers destroyed neurons but in each case D-HCA was the more powerful toxin. These findings strengthen the excitotoxic hypothesis and lend support to evidence from microelectrophoretic and ultrastructural studies suggesting that the receptor which mediates excitotoxic phenomena is on the external surface (dendritic or somal) of affected neurons. Although the premise that D-HCA is not transported intracellularly is based on indirect evidence (that D-HCA does not competitively inhibit active uptake of either L-GLU or L-HCA) it seems unlikely, in light of such evidence and all other available information, that either an intracellular uptake mechanism or an interference in intracellular metabolism underlies the excitotoxic activity of the HCA molecule (or of GLU and its other excitotoxic analogs since all members of the group are likely to have the same mechanism of excitotoxic action). Supported by USPH grants NS-09156, DA-00259 and RSD Award MH-38894 (JWO).

1657 THE EFFECT OF MITOCHONDRIAL UNCOUPLERS AND INHIBITORS ON EPINEPHRINE RELEASE FROM ADRENAL SECRETORY VESICLES. C.J. Pazoles, C.E. Creutz* and H.B. Pollard. NIH, Bethesda, MD 20014

Bovine adrenal chromaffin granules (CG) will release their contents when exposed to ATP, Mg and high concentrations of chloride salts (Hoffman et al. (1976) Arch. Biochem. Biophys. 176:375). Release is the result of an increase in the intragranular osmotic content and subsequent osmotic lysis of CGs (Pollard et al. (1976) JBC 251:4544). Recently we have shown that the osmotic content increase is due to an ATP stimulated influx of chloride ions through saturable anion transport sites in the granule membrane, and that anion transport blockers (SITS, probenecid, pyridoxal phosphate) inhibit release competitively with respect to external chloride ions (Pazoles and Pollard (1977) JBC, in press). However, the specific mechanism by which ATP promotes chloride transport is poorly understood. The CG possesses a Mg-ATPase and it has been suggested that this enzyme may play a role in release by pumping protons into the CG thus providing an electrical driving force for chloride uptake (Casey et al. (1976) Biochem. J. 158:583). The CG also possesses an electron transport chain which could be involved in the release reaction.

We have investigated the possible roles of ATPase and electron transport in the release reaction by studying the effects of mitochondrial uncouplers and inhibitors on release. The mitochondrial inhibitors oligomycin, antimycin-A and rotenone (all at 1.4 µg/ml) had no effect on release. Several uncouplers, however, were potent release inhibitors. These included TTFB (K_i = 0.15 µM), PCP (K_i = 2.5 µM), DNP (K_i = 30 µM), and FCCP. TFNB was not inhibitory. Kinetic analyses revealed that the uncouplers, like the anion transport blockers, inhibited release competitively with external chloride ions. We also found that the inhibitory uncouplers also stimulated the ATPase activity of CGs.

These results suggest that mitochondrial uncouplers, which are thought to act as proton ionophores, may actually inhibit release by interacting directly with the chloride transport site of the CG. These substances are, in fact, either anionic or strongly electronegative under the conditions employed. We conclude that although the stimulatory effect of uncouplers on the CG ATPase is consistent with its postulated proton pumping activity, this effect may be unrelated to the inhibition of CG release by these substances. Alternatively, it is possible that the ATPase and chloride transport sites are functionally coupled in a way which results in kinetic competition between uncouplers and chloride. Our results also indicate that the CG electron transport chain is either insensitive to mitochondrial electron transport inhibitors or is not involved in Mg-ATP-chloride induced release.

1658 ROLE OF PERMEANT ANIONS AND OSMOTIC LYSIS IN THE REGULATION OF EPINEPHRINE RELEASE FROM ISOLATED CHROMAFFIN GRANULES AND EXOCYTOSIS OF SEROTONIN FROM HUMAN PLATELETS. H.B. Pollard, C.J. Pazoles, C.E. Creutz and N.R. Shulman. NIH, Bethesda, MD 20014

Isolated chromaffin granules from bovine adrenal medulla release epinephrine when exposed to Mg^{2+}-ATP and high concentrations of permeant anions such as Cl^- (Hoffman, et al. (1976) Arch. Bioch. Biophys. 176:375). The release process depends on ATP-mediated, passive uptake of anions through an anion transport site and is competitively inhibited by impermeant, anion transport blocking agents, such as isethionate (Pollard et al. (1977) Prog. Clin. Biol. Res. 75:269), SITS (4-acetamido-4-isothiocyanostilbene -2-2'-disulfonic acid disodium), probenecid and others. The granule release event itself is due to osmotic lysis and was thus also suppressed by increased external osmotic strength (Pollard et al. (1976) JBC 251:4544). We have previously viewed this granule release reaction as a possible model for chemical events in exocytosis, and we now report that the anion transport blocking drugs also inhibited thrombin- and A23187-activated serotonin secretion from human platelets. The permeant anion of physiologic importance was hydroxyl ion rather than chloride, since serotonin release was unaffected by removal of Cl^- from the medium, and Dixon analysis (1/v vs [Inhibitor] at various pH's) showed the drugs to be competitive inhibitors with respect to OH^- concentration. K_i values were: suramin (0.9 µM); SITS (28 µM); pyridoxal phosphate (58 µM) and probenecid (335 µM). Serotonin secretion from platelets was also suppressed by increased osmotic strength of the medium, and the suppression curves, as a function of external osmotic strength, were identical for both the granule and platelet systems. We concluded that platelet secretion probably depended on anion transport and osmotic lysis analogous to those processes occurring in epinephrine release from granules, and that the general chemical and physical properties of the releasing chromaffin granule appeared to be conferred upon the secreting platelet. One explanation for this phenomenon could be that exocytosis in platelets occurs by serotonergic granules becoming "fused" or closely juxtaposed to the platelet plasma membrane, thereby allowing the granule's putative anion transport system access to OH^- ions in the external medium. The "fission" step of exocytosis might then occur as a consequence of osmotic lysis following anion entry into the vesicle interior. These findings may have fundamental importance for exocytosis in general, and could also lead to new clinical applications for anion transport blocking drugs.

1659 GABA AND NON-SYNAPTIC NEUROTRANSMISSION. E. Ramon-Moliner

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A selective action of GABA at synaptic sites appears quite likely (Takeuchi & Takeuchi, J. Physiol. 177:225,1965; Usherwood, Comp. Biochem. Physiol. 44A:663, 1973; Craelius, J. Physiol. 263:405, 1976). However, a second, extrasynaptic, type of action appears also likely. GABA acts on the 6th. abdominal segment of the crayfish, which lacks inhibitory innervation (Kufler & Edwards, J. Neurophysiol. 21:589, 1958). It depolarizes the posterior root ganglia (Nishi et al., Neuropharmacol. 13:215, 1974; De Groat, Brain Res. 38:429, 1972) in which no synapses of any kind are found (Andres, Z. Zellforsch. 55:1, 1961; Beaver et al., Arch. Path., 79:557, 1965; Pannese, Z. Zellforsch. 52:567, 1960; J. Comp. Neur. 132:331, 1968; Pannese et al., Brain Res. 46:215, 1972; J. Comp. Neur. 160:463, 1975). In the olfactory bulb, an inhibitory action of granule cells upon mitral and tufted neurons appears to be mediated by GABA (Nicoll, Brain Res. 35:137, 1971; McLennan, Brain Res. 29:177, 1971). These granule cells appear to be filled with vesicles of post-synaptic nature (Ramon-Moliner, Brain Res. 105:551, 1976) and devoid of efferent synapses (Ramon-Moliner, Brain Res., in press). GABA appears to be involved in presynaptic inhibition (Eccles et al., J. Physiol. Lon. 168:500, 1963; Davidoff, Science, 175:331, 1972) but axo-axonic synapses are very rarely observed in the spinal cord of frog (Glusman et al., Neurosci. Let. 2:137, 1976) or of the cat (Ramon-Moliner, unpublished observations). Therefore, a probable non-synaptic interaxonic modulation is involved in the mechanism of presynaptic inhibition and primary afferent depolarization, the latter being influenced by drugs that interfere with the GABA system (Barker and Nicoll, J. Physiol. Lon. 228:259, 1973; Davidoff, Exp. Neurol. 35:179, 1972). The possible involvement of glia should be considered: it is rich in GABA (Neal and Iversen, Nature, 235:217, 1972; Ljungdahl and Hökfelt, Brain Res. 62:587, 1973; Minchin and Iversen, J. Neurochem. 23:533, 1974; Iversen and Kelly, Biochem. Pharmacol. 24:933, 1975; Hammerstad and Lytle, J. Neurochem. 27:399, 1976) and it could release this transmitter as a result of the accumulation of extracellular potassium (Young et al., Brain Res. 63:479, 1973) following axonal depolarization.

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1660 CATECHOLAMINE-STORING GRANULES FROM A CLONED CELL LINE. R. Victor Rebois* and Bruce D. Howard (SPON: S. Eiduson) UCLA Medical School, Los Angeles, CA 90024.

Greene and Tischler (PNAS 73:2424, 1976) have established a pheochromocytoma cell line (PC12) from rat adrenal medulla. These cells store catecholamines and exhibit a depolarization-induced, Ca^{2+}-dependent release of catecholamines (Trans. Amer. Soc. Neurochem. 8:161, 1976). We have found that these cells are a good source of catecholamine-containing storage granules. These granules are similar to chromaffin granules from normal adrenal medulla and to catecholamine-containing synaptic vesicles with respect to their mechanism of storage of catecholamines. Storage granules from PC12, like chromaffin granules and catecholamine-containing synaptic vesicles, accumulate catecholamines by an ATP-stimulated process. For these studies cells were removed from culture plates by washing with growth media, pelleted by centrifugation, and homogenized in a Teflon-glass tissue grinder. Storage granules were isolated by a series of centrifugation steps. The granules were preincubated for 5 min at 30° and then with 0.1 μM [^{3}H]dopamine (16 Ci/mmol) for an additional 5 min in a 10 mM phosphate buffer (pH 7.4) containing 0.32 M sucrose, 1 mM Mg^{2+} and 1 mM ATP. The accumulated dopamine was separated from the medium dopamine by passage through a small column of Sephadex G-25. Under these conditions, approximately 30 pmoles of dopamine were accumulated per mg of granule fraction protein. Dopamine uptake was stimulated by ATP and inhibited by 0.2 μM reserpine. The ATP-stimulated uptake was also inhibited by 1 μM S-13, an uncoupler that dissipates proton gradients, and by 40 μM dicyclohexylcarbodiimide (DCCD), which inhibits some Mg^{2+}-ATPases, but not by ouabain, which inhibits Na^+/K^+-ATPase activity. The granules contain a Mg^{2+}-ATPase activity that was inhibited by DCCD. These results indicate that catecholamine uptake by storage granules from PC12 cells is driven by a pH gradient that in turn is generated by a Mg^{2+}-ATPase activity associated with the granules. Thus, the energetics of catecholamine uptake by PC12 storage granules is similar to that of chromaffin granules from normal adrenal.

1661 LITHIUM: EFFECTS ON THE ELECTROGENIC SODIUM PUMP AND SLOW SYNAPTIC INHIBITION. Peter A. Smith* and Forrest F. Weight. Lab. Neuropharm., NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032

The cellular mechanisms of the action of lithium in the nervous system is of interest because of its importance in treating certain types of mental illness. We tested the effect of lithium on the electrogenic sodium (Na) pump and the slow IPSP in bullfrog sympathetic ganglia using the sucrose gap technique. The administration of acetylcholine (ACh: 3 mM) produces a nicotinic depolarization followed by an after-hyperpolarization (AH) that results from the activation of the electrogenic Na pump. When Na in the Ringers solution was replaced by Li, the ACh depolarization still occured but the AH was abolished. Presumably Li is not extruded by the electrogenic Na pump, as has been found previously in other tissues. Li-Ringer also blocked the muscarinic slow IPSP and the hyperpolarizing response to agonists such as methacholine. We recently found that ouabain and K-free Ringer can effectively inhibit the electrogenic Na pump without blocking the slow IPSP (Nature 267 68, 1977), indicating that the slow IPSP is not generated by activation of the electrogenic Na pump. In view of this, we conclude that the effect of lithium on the slow IPSP is not a direct consequence of sodium pump inhibition.

1662 THE ROLE OF Mg^{2+}-ATPase AND A pH GRADIENT IN THE STORAGE OF CATECHOLAMINES IN SYNAPTIC VESICLES. Lawrence Toll* and Bruce D. Howard. UCLA Medical School, Los Angeles, CA 90024.

We have obtained evidence that a Mg^{2+}-ATPase situated on the membrane of catecholamine-storing synaptic vesicles is utilized in the loading of catecholamines into the vesicles. This ATPase appears to be a proton-translocating enzyme. Its activity generates a transmembrane pH gradient that in turn drives catecholamine transport. A similar mechanism can be invoked for the loading of catecholamines into adrenal chromaffin granules. For this study we have measured the in vitro, ATP-stimulated uptake of [^{3}H]norepinephrine (NE) by a fraction enriched in synaptic vesicles from rat brain. Both the uptake of NE and the Mg^{2+}-ATPase activity were inhibited by 40 μM dicyclohexylcarbodiimide (DCCD), 3 μM tributyltin and 100 μM 4-chloro-7-nitrobenzofurazan (Nbf-Cl), which are known also to inhibit proton-translocating ATPases from other sources. Catecholamine uptake was inhibited by 100 μM dinitrophenol (DNP), 4 μM FCCP and 1 μM S-13, which are known to make membranes permeable to protons. Johnson and Scarpa (J. General Phys. 68:601, 1976) and others have shown that the internal pH of chromaffin granules is approximately 5.5, indicating a transmembrane ΔpH of almost 2 units. They demonstrated that this proton gradient could be dissipated by the utilization of several cation ionophores. We have found that the ability of these ionophores to inhibit NE uptake by synaptic vesicles corresponds well with their reported ability to dissipate the pH gradient in chromaffin granules. FCCP at 0.4 μM and the K^+ ionophore valinomycin at 3 μg/ml individually had only a small effect on NE uptake or on the ΔpH, but acting together they greatly inhibited uptake and markedly altered the pH gradient. The Ca^{2+} ionophore A23187 was shown to dissipate the pH gradient only in the presence of Ca^{2+} or Mg^{2+}; A23187 was a more effective inhibitor of NE uptake in the presence of 1 mM Ca^{2+}, 1 mM Mg^{2+} than in the presence of 1 mM Mg^{2+}. Finally, nigericin, which catalyzes a K^+/H^+ exchange greatly inhibited NE uptake.

The effect of various compounds on NE efflux was studied by incubating [^{3}H]NE-loaded synaptic vesicles with the compounds. Compounds that inhibit uptake by acting at the ATPase had little or no effect on efflux. Also 0.2 μM reserpine, which inhibits NE uptake but does not affect the pH gradient, did not cause efflux. But, all the compounds that dissipated the transmembrane pH gradient also caused a significant efflux of NE from the vesicles. These results indicate that a pH gradient is indeed involved in the loading of NE into synaptic vesicles and in the maintenance of at least some of the freshly accumulated transmitter within the vesicles. It remains to be determined whether a membrane potential also is involved in the storage of NE in the vesicles.

1663 UNEXPECTED BIDIRECTIONAL CROSS TOLERANCE TO THE EFFECTS OF ETHANOL AND LOW TEMPERATURE ON THE RATE OF PTP DECAY AT AN IDENTIFIED SYNAPSE IN APLYSIA. M. Elaine Traynor*, Werner T. Schlapfer, Paul B. J. Woodson and Samuel H. Barondes. Depts. of Neurosciences and Psychiatry, UCSD, La Jolla, CA 92093, and Dept. of Psychiatry, VAH, San Diego, CA 92161.

The rate of decay of post-tetanic potentiation (PTP) at the identified synapse, RC1-R15 is accelerated by perfusion with 0.8 M ethanol (Nature 260: 797, 1976) and decelerated by reduction of the temperature (Nature 258: 623, 1975). There is a sharp transition in the temperature dependence of the rate of PTP decay in going from 12 to 10°C; such that the rate of PTP decay is more markedly affected over this interval than over neighboring intervals (Nature 258, op cit.). The effects of ethanol and low temperature both show adaptations that we refer to as "tolerance." Repeated exposure to ethanol results in a tolerant state in which ethanol no longer accelerates PTP decay (Science 193: 510, 1976). Likewise, keeping the preparation below 10°C for 4 or more hours leads to either a shift to a lower temperature, or a disappearance of the transition in the temperature dependence of the PTP decay rate, although PTP still decays more slowly at low temperatures (Nature 258: 623, 1975). Such a preparation is said to be temperature adapted.

We now report that preparations which are tolerant to ethanol do not exhibit a transition in the temperature dependence of the PTP decay rate; i.e. they appear to be temperature adapted (n = 4). Conversely, preparations which are temperature adapted are tolerant to the effect of ethanol in accelerating PTP decay rate (n = 7). Preparations which were treated in all ways similar to ethanol tolerant preparations, except for exposure to ethanol, showed a sharp transition in the PTP decay rate upon going from 12 to 10°C (n = 2). Preparations which were treated in all ways similar to temperature adapted preparations, except for exposure to temperatures below 15°C showed a marked acceleration of PTP decay rate by 0.8 M ethanol (n = 5).

The finding of bidirectional cross tolerance to treatments with opposite actions is unexpected. Further experiments, in progress, may help explain it.

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1664 BENZODIAZEPINES MIMIC THE PRESYNAPTIC ACTION OF GABA ON A CHOLINERGIC SYNAPSE OF APLYSIA. J.-P. Tremblay and G. Grenon*. Lab. Neurobiol. Fac. Med. Laval Univ. Quebec, QUE. Canada.

The concept that benzodiazepines act through a GABAminergic mechanism has been recently developed by Costa et al., (Life Sci. 17, 167, 1975). Benzodiazepines have also been shown to affect brain acetylcholine level (Consolo et al., Eur. J. Pharmacol. 27, 266, 1974; Domino and Wilson, Psychopharm. 25, 291, 1972).

The effects of two water soluble benzodiazepines (flurazepam and RO-5-6901) have been studied on a monosynaptic, unitary and cholinergic synapse recorded in cell R15 of Aplysia after minimal stimulation of the right connective. Trains of 100 stimuli at 1/sec, followed by test pulses at interval ranging from 20 to 200 sec were given every 10 to 30 min (variable in different preparation). At this frequency, the size of the first few EPSPs decreases (synaptic depression), but with further stimulation the EPSP size increases to a sustained facilitated plateau (frequency facilitation). The train is followed by a period of post-tetanic potentiation (PTP) during which EPSPs are even larger than during the facilitated plateau.

Addition to the perfusate of a benzodiazepine (10^{-5} to 2×10^{-4} M) reduces the size of all EPSPs of a train. The EPSP size reach about 50% of their control value in 1 to 3 hrs depending on the drug concentration used. The benzodiazepines also reduce the synaptic depression and the PTP and increase frequency facilitation ratio (size of hundredth EPSP of a train divided by the size of the first EPSP of that train). The membrane potential was slightly depolarized (0 to 12 mV, N = 10) and there was a slight (0 to 20%) reduction of the membrane resistance.

The effects of the benzodiazepines were attributed to a presynaptic action since the slight changes in membrane resistance and potential of the postsynaptic cell could not account for the large reduction of EPSP size, and for the changes of synaptic depression, frequency facilitation and PTP. These effects of benzodiazepines are similar to those previously observed with GABA at this synapse (Tremblay and Plourdes, Can. J. Physiol. and Pharmacol., submitted) and they are probably due to a direct or indirect action on a presynaptic GABA receptor. Benzodiazepines have also been previously observed to reduce PTP of another cholinergic synapse in the bullfrog sympathetic ganglia (Suria, Neuropharm. 15, 11, 1976).

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1665 MODULATION OF TRANSMITTER RELEASE AT AN INHIBITORY SYNAPSE IN THE C.N.S. OF THE LEECH. Bruce G. Wallace* and John Nicholls. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

An inhibitory synapse that exhibits several unusual features has recently been described by Thompson and Stent (1976). In particular they observed that as the membrane potential of the presynaptic neuron became depolarized, each of its impulses gave rise to a larger inhibitory potential in the postsynaptic neuron. (This is the opposite of the conventional picture of presynaptic inhibition). We have now investigated the properties of this synapse in greater detail, measured the effect of presynaptic depolarization and hyperpolarization on the quantum content of synaptic potential and shown in addition that presynaptic inhibition at this synapse results from hyperpolarization of the terminals.

The synapse is situated in the third most anterior ganglion in the nerve cord, between an inhibitory interneuron and a motoneuron that supplies the heart. The relationship between the membrane potential in the presynaptic neuron and the amount of transmitter released by each impulse is steep. For example depolarizing the presynaptic cell body by 10 mV increases the size of the inhibitory potential recorded in the postsynaptic cell by a factor of about 6. This increase in release develops gradually over the course of about 0.5 sec after a step depolarizing change in membrane potential and decays slowly after a hyperpolarizing step. Unlike other synapses described in the leech, when the presynaptic cell is stimulated to give trains of impulses at various frequencies little facilitation or depression is apparent.

Presynaptic inhibition of release occurs by way of the homologous interneuron on the contralateral side of the ganglion. Its impulses produce inhibitory potentials on its homologue (i.e. the presynaptic interneuron). These hyperpolarizations reduce the sizes of inhibitory potentials recorded in the heart motoneuron. Recordings made from the interneurons on both sides of the animal show that both modulation of release and presynaptic inhibition occur as normal physiological events in generating the rhythmical activity of the motoneurons that supply the heart. Under normal circumstances, the interneurons fire out of phase with one another: thus the bursts in one cell inhibit the other and depress the amount of transmitter it releases.

Thompson, W. J. & Stent, G. S. (1976). J. Comp. Physiol. 111, 309-333.

1666 ENDOGENOUSLY GENERATED PTP AT AN IDENTIFIED SYNAPSE IN APLYSIA AND ITS MODULATION BY PERIPHERAL TONICITY SENSORS. Paul B. J. Woodson and Werner T. Schlapfer. Depts. of Psychiatry, UCSD, La Jolla, CA 92093 and VAH, San Diego, CA 92161.

Kupfermann and Weiss (J. Gen. Physiol. 67: 113, 1976) suggest that cell R15 of the abdominal ganglion of Aplysia releases a hormone which causes weight gain, probably through supression of water secretion. In the absence of synaptic input, R15 is a spontaneous burster (Strumwasser, J. Psychiatr. Res. 8: 237,1971). We now report that in both, an intact preparation (restrained unanesthetized animal with externalized abdominal ganglion), and a semi-intact prep (connected tentacular, circumeosophageal, abdominal, branchial, osphradial ganglia, anterior tentacles and osphradium), the bursting of R15 is strongly modulated by patterned bursts of the large EPSP which we call RC1-R15 (Brain Res. 109: 1, 1976). The membrane properties of R15 confer a tendency to fire in bursts during RC1-R15 maintained depolarization. In both preparations, the spontaneous firing of RC1-R15 may be tonically accelerated or decelerated by hyper- or hypotinic seawater, respectively, applied to the anterior tentacles. This sensory input does not habituate. Lifting the anterior tentacle out of the water, drying it off, leads to acceleration of the firing of RC1-R15. Scratching, poking or applying amino acids implicated in sexual or feeding behavior to the anterior tentacles do not affect RC1-R15 firing rate. The normally high spontaneous firing rate of RC1-R15 (intraburst frequency reaches 10/sec, with bursts a few seconds to many mins. apart) keeps the EPSP in a state of post-tetanic potentiation, which decays slowly when spontaneous firing is stopped by a sucrose gap on the right connective (presynaptic axon). In the blocked prep, electrical stimulation of the right connective reveals the decay of PTP. When PTP has decayed, the EPSP amplitude may be PTP'd either artificially by repetitive stimulation of the right connective (Brain Res., op. cit.) or by removal of the sucrose block.

We conclude that the efficacy of RC1-R15 reflects the tonicity of the environment both in its firing rate and in its amplitude because of the dependence of the PTP mechanism on the presynaptic firing rate. The PTP component of this synapse's efficacy appears to be regulated by presynaptic serotonin receptors (Newlin, et al., this volume) and by a heterosynaptic input (Brain Res. 109: 83, 1976).

1667 COMPARISON OF EXCITATION OF SINGLE CORTICAL NEURONS IN AWAKE CATS BY EXTRACELLULARLY AND INTRACELLULARLY DELIVERED CURRENT. C. D. Woody and E. Gruen*. UCLA Med. Center, Los Angeles, Ca. 90024.

Multiple barrel, glass microelectrodes were used to deliver current while recording intracellularly from neurons of the coronal peri-cruciate cortex. Baseline potential shifts on penetration averaged 45mV. The electrodes were separated vertically at the tips by 10-20μ to permit separate, simultaneous intracellular (IC) and extracellular (EC) recordings from the same unit. EC recordings did not have significant baseline shifts. After establishing absence of significant crosstalk between barrels, effects of constant current delivery via the IC and EC barrels were compared. Rectangular cathodal (+) current pulses of 10 msec duration were used to excite, sometimes with superimposed steady intracellular depolarizing or hyperpolarizing currents.

The preliminary studies, conducted in 28 neurons, gave consistent results. IC currents of 0.4 - 2.0nA produced repeated spike initiation during current application in all units. EC currents of 2-100nA promoted discharge in all but five neurons. Other details on neural excitation with weak EC currents are given elsewhere (Woody et al. J. Neurophysiol. 1976). The passage of steady depolarizing IC current facilitated repeated spike initiation with extracellularly delivered pulses. The passage of steady hyperpolarizing IC current prevented repeated spike initiation with the extracellularly delivered pulses.

Reversible decreases in spike height were observed with the passage of depolarizing currents, intracellularly, but not with delivery of extracellular currents, even though they were as much as 50-100 times greater. No indications that extracellularly applied currents of these magnitudes excited by depolarizing the inside of the neuron towards critical firing threshold were obtained from recordings with intracellular electrodes close enough to the regions of spike initiation to pass weak intracellular currents sufficient to excite the cell or alter its response to the extracellularly applied currents.

Repeatable PSP activity was not detected when repeatable spike initiation by extracellular current was prevented by superimposed, steady hyperpolarizing current, delivered intracellularly. This indicates that the EC current did not excite through pre-synaptic terminals via PSP's produced by chemical neurotransmission.

Apparently, weak EC nA currents can facilitate unit excitation by means other than internal depolarization or PSP production. Earlier studies using EC nA stimulation (Woody et al. op cit.; Brons et al. Fed. Proc. 1977) indicate that currents of either polarity can frequently excite at equal strengths, although positive polarities are preferred, and that variations in amounts of current required to excite as a function of behavioral state are a consequence of altered neurotransmission. Supp. by BNS76-06886.

TISSUE CULTURE

1668 REGULATION OF ARYLSULFATASE A AND B IN CULTURED MOUSE NEUROBLASTOMA. V. J. Aloyo, S. J. Tarnowski* and S. V. Molinary. Dept. Biochem., UTCHS, Memphis, TN 38163.

The differentiation of mouse neuroblastoma C1300 is accompanied by a variety of biochemical and electrical changes as well as neurite formation. We show below that in clone N18 the specific activities of arylsulfatases A (ARA) and B (ARB) are differentially regulated by culture conditions.

The cells were grown in Falcon Tissue culture flasks in Dubbecco's Modified Eagle's Medium supplemented with 10% Fetal Calf Serum (FCS) at 37°C in an atmosphere of 10% CO_2 & 90% air. To initiate the experiments, the log phase cells were dislodged from the flasks and planted in media containing a final concentration of 1% or 10% FCS. Forty eight hours later the media were exchanged with fresh media containing either 0, 1 or 10% FCS. At specific times after planting, the cells were harvested by incubation at 37°C for 10 min. in versene. Harvested cells were diluted to approximately 2 x 10^6 cells/ml with distilled water. Sonication with intermittent cooling periods was used to rupture lysosomal membranes. Assays for ARA, ARB and protein were conducted on aliquots of the sonicate.

During log phase growth, the specific activity of ARA in the undifferentiated cells remained unchanged; the more differentiated cells (grown in 1% FCS) showed a small increase in specific activity. When the growth rate was decreased by reaching stationary phase or reducing the concentration of FCS (1% or 0%), increased differentiatiation of the cells occurred and the specific activity of ARA increased approximately two-fold.

In contrast, during log phase the specific activity of ARB increased 2 fold for the cells grown in either 1 or 10% FCS. The differentiated cells (grown in 1% FCS) showed a higher specific activity. Increased differentiation (and reduced growth rate) did not result in any further increase in the specific activity of ARB.

These results indicate that the arylsulfatases are independently altered with respect to cell differentiation or growth.

1669 ACETYLCHOLINE SYNTHESIS AND STORAGE BY SPINAL CORD NEURONS GROWN IN DISSOCIATED CELL CULTURE WITH MUSCLE. Darwin Berg. Dept. Biol., UCSD, La Jolla, CA 92093.

Many dissociated spinal cord neurons from 4 day old chick embryos form cholinergic synapses on muscle when grown in cell culture. The studies reported here examined the synthesis and storage of acetylcholine (ACh) by spinal cord cells in order to follow the development of cholinergic neurons in culture and to determine what factors influence the availability of neurotransmitter at cholinergic synapses.

Dissociated spinal cord cells from 4 day old embryos were grown on pre-plated muscle cultures. ACh synthesis was measured by incubating cultures in a balanced salt solution (BSS) containing (^{3}H)choline; cell extracts were prepared and assayed for (^{3}H)ACh by high voltage paper electrophoresis and scintillation counting. ACh production was linear with time for periods up to 1 hour and was proportional to the number of spinal cord cells initially added(1-12x10^5/35 mm dish). High choline concentrations (100 µM) were needed to achieve maximum rates of synthesis. Values of 30 and 400 pmoles/hr/mg protein were obtained at 0.5 and 100 µM choline for 8 day old spinal cord-muscle cultures seeded with 4x10^5 spinal cord cells. At all choline concentrations (0.5-100 µM) 10-20% of the total radioactivity accumulated in a 1 hour period was present as (^{3}H)ACh. Pre-incubation of cultures for 1 hour in BSS lacking choline or containing 75 mM K+ (to depolarize the cells) had no appreciable effect on the amount of ACh synthesized or total radioactivity accumulated by the cells.

Studies on the stability of intracellular ACh distinguished two pools. Spinal cord-muscle cultures were incubated in 1 µM (^{3}H)choline for 1 hour to label internal ACh stores. The cultures were rinsed, transferred to BSS containing 10 mM unlabeled choline, and analyzed at appropriate times. About 40% of the (^{3}H)ACh present was lost during the first 3 minutes of the chase period. The remainder was depleted with a half-time of *ca.* 0.5 hr. Thus at least a sub-population of chick embryonic spinal cord neurons can use exogenous choline to synthesize ACh and can store a large fraction of the ACh in a relatively stable pool.

Development of the cholinergic neuron population as monitored by capacity for ACh synthesis proceeded in a continuous manner. Rates of synthesis measured either at 1 µM or 50 µM choline increased linearly with culture age over a 2-1/2 week period. Spinal cord cells grown for 1 week on collagen-coated dishes in the absence of muscle synthesized ACh at rates comparable to those observed for cells grown with muscle. By this criterion post-synaptic muscle cells were not necessary for the initial stages of development of the cholinergic neurons. (Supported by USPHS Grant #12601-01, Muscular Dystrophy Assoc., & Amer. Heart Assoc.)

1670 CARBACHOL DOWN REGULATES ACETYLCHOLINE RECEPTORS IN MOUSE MUSCLE CELL CULTURES. Thomas H. Brown, Mark Noble* and John H. Peacock. Dept. Neurol., Stanford Univ. Sch. Med., Stanford CA 94305.

Acetylcholine (ACh), in its role as a depolarizing agent mediating neurotransmission, is important for down regulation of ACh receptor levels in skeletal muscle in situ. However, there have been no successful attempts to down regulate the number of ACh receptors by continuous application of ACh to denervated, isolated muscle (1). Indirect evidence from in vivo work (2), where synaptic cleft ACh is increased after AChesterase block, supports the argument that ACh down regulates its receptor, but is subject to alternative interpretations. In the present experiments we demonstrate directly that carbachol, a stable ACh analogue, produces a substantial down regulation of receptor levels in noninnervated muscle cell cultures after a 2-5 day incubation period. Cultures were prepared from the G8 mouse myoblast line which has high receptor levels and shows little or no desensitization to prolonged ACh application. Effective carbachol concentrations (10^{-3}-10^{-4}M) were somewhat lower than estimates (3) of peak endogenous ACh concentration at the neuromuscular junction (3x10^{-4}M).

Data from three methods used to measure ACh receptor levels are in agreement. (i) Specific binding of ^{125}I-α-bungarotoxin is decreased by 30-50% (Table). This reduction is not due to increased receptor degradation rates or desensitization. (ii) Maximum sensitivity to iontophoretically applied ACh (Table) is decreased by about 70%. ACh sensitivity was evaluated in blind experiments 1-6 hours following a control medium change to eliminate any possible desensitization. (iii) Autoradiography shows decreased labeling of both focal receptor patches and diffusely distributed receptor. The results demonstrate directly that a nicotinic agonist can down regulate ACh receptor levels and offer another approach to understanding such regulatory mechanisms.

CONDITION	BINDING fmol toxin/mg protein ± SE(n)	RESTING MEMBRANE POTENTIAL mv ± SE(n)	MAXIMUM ACh SENSITIVITY mv/nC ± SE(n)
Carbachol (10^{-4}M)	4.9 ± 0.21(8)[a]	47.3 ± 1.1(34)[c]	834 ± 198(34)[c]
	8.3 ± 0.56(8)[b]		
Control	8.1 ± 0.35(8)[a]	47.0 ± 1.2(24)[c]	3042 ± 386(24)[c]

Incubation periods: (a) 2 days, (b) 20 min, (c) 3-5 days

1. Miledi, R (1960) J PHYSIOL 151:1.
2. Chang, CC, Chen, TF, & Chuang, ST (1973) J PHYSIOL 230:613.
3. Kuffler, SW & Yoshikami, D (1975) J PHYSIOL 251:465.
(Supported by NIH grant NS12151 to J.H.P.)

1671 LOCAL CONTROL OF NEURITE GROWTH AND SURVIVAL BY NERVE GROWTH FACTOR. Robert B. Campenot (SPON: Paul H. Patterson). Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

A tissue culture system has been devised in which dissociated rat sympathetic neurons grown on a collagen surface in liquid medium send their neurites across a barrier into a separate compartment. Although the barrier seal is permeable to the growing neurites, it is sufficiently impermeable to medium to hold a 5 mm hydrostatic head for at least 4 days without appreciable equalization. This culture system makes it possible to study the effects of chemical gradients on neurite growth and the effects of local application of substances to either the neurite endings or the somas.

Sympathetic neurons plated into medium containing nerve growth factor (NGF, 1 µg/ml) regularly sent neurites across the barrier into compartments which also contained NGF, but neurites never entered compartments to which no NGF had been added. This result shows that the local NGF concentration to which the growth cone is exposed can control the direction of neurite growth.

Experiments in which NGF was withdrawn from chambers after the neurites had entered suggested that local NGF is also essential for neurite survival. The neurites degenerated after local NGF withdrawal even though other portions of the neurons, including the somas, had continuous access to NGF. In contrast, the somas were not dependent on local NGF. Many somas survived withdrawal of local NGF, provided they were associated with neurite bundles that had crossed into compartments containing NGF. All the neurons in the same cultures which clearly had not sent neurites across the barrier degenerated. In summary, it seems that regions of a sympathetic neuron retrograde from a local NGF source are maintained, whereas regions orthograde from the source degenerate.

The results of these experiments are consistent with the notion that NGF released by sympathetic target tissues plays an important role in establishing and maintaining appropriate neuron-target connections during development.

1672 **IN VITRO DEVELOPMENT OF ENTERIC GANGLIA.** **H. Cheng* and M. Bjerknes* (SPON: R. E. Stull). School of Basic Medical Sciences, University of Illinois, Urbana, ILL 61801.**

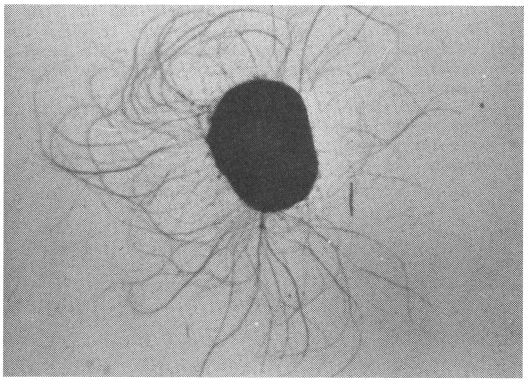
The intestine from 15-day old White Leghorn chicken embryos was removed and placed in iced-cold sterile Tyrode's solution. The mesentery was then removed and the intestine minced with fine scissor. The explants were plated in 60 mm Falcon tissue culture dishes which had been pretreated with 2% gelatine and incubated at 37°C in an atmosphere of 5% CO_2 and 100% humidity. The medium (modified Waymouth MB 752/1 with 10% fetal calf serum, 50 U/ml of penicillin and 50 ug/ml of streptomycin) was changed every week. Outgrowth (fibroblastic or epithelial) from intestinal explants was observed as early as 24 hours after initiation of culture. In a week's time ganglia consisting of various numbers of large neurones can be observed to develop in or near the explant. Ramification of nerve processes (some well over 1 mm in length) from these neurones was soon observed followed by appearance of large nerve bundles. Nerve processes have been observed to terminate on cells or end free in the medium. Nerve bundles were often found connecting two ganglia. With fluorescence microscopy after drying over phosphorus pentoxide and exposure to formaldehyde vapour, both the ganglia and nerve bundles exhibit yellowish green fluorescence indicating their adrenergic nature. Such cultures have been maintained for over 45 days and in no cases was nerve growth factor added. These results demonstrated development of enteric ganglia in vitro from chicken intestine. (Supported by a grant from The National Foundation-March of Dimes.)

1673 A FIXED-ARRAY, GOLD MULTI-MICROELECTRODE SYSTEM FOR LONG-TERM SIMULTANEOUS RECORDING OF SINGLE UNIT ACTIVITY IN VITRO. Guenter W. Gross*, Ellen Rieske*, Andreas Meyer* & Alfred Polityckí* (SPON: Paul Cancalon). Exp. Neuropath. Sect., Max Planck Inst. for Psychiatry 8 Munich 40, and Forschungslaboratorien München der Siemens AG, 8 Munich 80, FRG.

We have developed a new high density, fixed-array multi-electrode system for long-term monitoring of extracellular neuronal activity in vitro. The electrode system is manufactured with conventional microelectronics and new laser microbeam methods. It consists of a 4x4 cm glass plate that serves as the floor of a culture chamber and carries 36 gold conductors (10 μm wide and 2 μm thick). The conductors start at contact strips at either side and terminate in a centered matrix of 6 rows (200 μm spacing) and 6 columns (100 μm spacing). The conductors are insulated by the application of a silicone resin to the electrode plate spinning at 2500 rpm. This yields a 1 to 2 μm thick insulation layer that is then increased to 100 μm over the conductor bands outside the 0.5 x 1 mm recording area. In this manner the shunt impedance between the culture medium and the gold conductors is maintained above 300 megohms at 1 kHz and signal attenuation is minimized. The electrode tips are de-insulated by utilizing a UV laser microbeam system (BTG Biotechnik, Munich). Highly reproducible insulation breakthrough is accomplished with a single 8 nanosecond laser pulse. This is carried out in saline so that the impedance can be monitored during tip production. Impedances of 5 to 30 megohms can be produced by varying the area of the exposed metal surface from 100 to 10 μm^2. Preliminary testing of the electrode system with excised ganglia of the snail Helix pomatia has demonstrated that single unit activity can be easily obtained from a variety of sizes of neurons. Spikes of 250 μV can be recorded from submerged ganglia that are resting against the recording matrix. Insulation layers are stable for several weeks if large and sudden temperature variations are avoided. Insulating materials that allow convenient sterilization and promote cell adhesion are presently being tested.

1674 CLOCKWISE GROWTH OF NEURITES FROM RETINAL EXPLANTS. Anne M. Heacock and Bernard W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, Michigan 48109.

Goldfish retinal explants exhibit a vigorous neuritic outgrowth when cultured on a collagen-derived substratum, provided the optic nerve had been crushed one to two weeks prior to explantation. On the fibrous collagen surface, the neurites are randomly oriented and show no obvious directionality. We report here that polycation-coated glass or plastic surfaces also support neuritic outgrowth from goldfish retinal explants, but that on such surfaces, the neurites show a marked tendency to curve in a clockwise direction. Curving neurite outgrowth is observed both on surfaces coated with synthetic polycations such as poly-L-lysine, and on those coated with naturally occurring polycations such as protamine. The clockwise directionality is not imparted by the chirality of the poly-L-lysine or protamine substrata or by external guidance factors such as electromagnetic, gravitational or Coriolis force fields. A relationship of the clockwise pattern to an anatomical frame of reference within the explant or derived from the donor was also ruled out. We conclude that the observed clockwise directionality is somehow a consequence of an inherent helicity of the neurites which is expressed as the growing tip advances on a suitable adhesive planar surface. The phenomenon may have some relevance to the mechanisms by which retinal fibers find their way to selected sites in the brain in a highly ordered fashion. (Supported by PHS MH12506.)



1675 REGENERATION OF RETINAL GANGLION CELL AXONS IN VITRO. Pamela R. Johns, Anne M. Heacock, and Bernard W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, Michigan 48109.

We previously reported that neurites grow out from explants of goldfish retina, and we suggested that these neuritic processes are the regenerating axons of the retinal ganglion cells. This inference was based on indirect evidence: 1) only ganglion cells have long axons which extend outside the retina in vivo, and 2) the neuritic outgrowth is more vigorous and rapid when the optic nerve is crushed 10-14 days prior to explantation.

In the experiments reported here we took advantage of the laminar arrangement of the retinal neurons to trace the neurites to their cells of origin using light and scanning electron microscopy. Full thickness retinal explants were prepared either as 500 μm squares or as 100 x 400 μm strips. The strips were oriented cut side up so that the retinal layers were seen in "cross section," whereas the larger, square explants were placed with either vitreal or photoreceptor surface up and viewed as "flat mounts." Holmes silver staining was used to visualize the retinal neurons and their processes. Many of the neurites outside the explant were contiguous with the parallel array of axons in the optic fiber layer within the explant. In instances in which individual neurites could be traced back to their cells of origin, the perikarya invariably exhibited the characteristic morphology of retinal ganglion cells and were found in the appropriate retinal layer. Neurites were also traced to ganglion cells in explants examined by scanning electron microscopy. In addition, light microscopic examination of the retinal explants revealed that most or all of the retinal cells other than the ganglion cells had degenerated after three weeks in culture.

From these observations we conclude that the neurites are indeed ganglion cell axons. Explant culture of adult goldfish retina therefore provides a model system for the study of optic nerve regeneration. (Supported by PHS NS05518 to P.R.J. and MH 12506 to B.W.A.)

1676 CHEMICAL CHARACTERIZATION OF GLIA MATURATION FACTOR. Taiji Kato*, Shuang S. Troy*, David E. Turriff and Ramon Lim. Brain Research Institute, University of Chicago, Chicago, IL 60637

Glia maturation factor (GMF), a protein that promotes the morphological and chemical maturation of glioblasts in culture, was identified in our laboratory in the adult brain and some other organs (Science, 185:63-66, 1974; Science, 195:195-196, 1977). The physicochemical and biological properties of GMF distinguish it from other related factors such as nerve growth factor (NGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF).

Recently we have worked out a new isolation technqiue for the purification of GMF from lyophilized crude pig brain extracts by means of successive chromatography through sephadex G-200, DEAE sephadex and sephadex G-150 columns. With this method the native protein (MW.350,000) is dissociated into a smaller component (MW. 40,000) which remains the biological activity.

Partial characterization of GMF indicates that: (i) it is sensitive to pronase and resistant to trypsin, but there is no enhancement of activity by trypsin treatment as would have been expected in an enzyme-proenzyme system; (ii) it is sensitive to heating at 100°C but stable in 4M urea; (iii) its isoelectric point is slightly acidic; (iv) using potentiometric and spectrophotometric assay, GMF has neither esterase activity on N-Benzoylarginine-ethylester , N-Benzoyltyrosine-esthylester and Tosylarginine methylester substrate, nor amidase activity on N-Benzoylarginine-p-nitroamilide substrate; (v) reducing reagents such as Dithiothreitol, alkylating reagents such as iodeacetamide, and reagents for chemical modification of active serine (Diisopropyl-fluorophosphate) do not destroy or reduce the biological activity of GMF. Attempts at obtaining specific antibody against GMF is currently underway. (This work was supported by U.S. Public Health Service Grants No. NS-09228, CA-19266 and NS-07376)

1677 MEMBRANE PROPERTIES OF A HUMAN NEUROBLASTOMA; EFFECTS OF DIFFERENTIATION. T. Kuramoto, J.R. Perez-Polo and B. Haber, (Spon. S. Wolf) The Marine Biomedical Institute, Dept. of Human Biol. Chem. and Genetics, and Dept. of Neurology, University of Texas Medical Branch, Galveston, Texas 77550

The SK-N-SH cell line is a human neuroblastoma (J. Biedler) which when grown under standard culture conditions remains relatively undifferentiated, and has only short processes. We have previously shown that the undifferentiated SK-N-SH cells are relatively inexcitable; they show only partial active responses to injections of current and appear to lack the depolarizing component of the action potential generating mechanism. The relative inexcitability of these cells as determined by intracellular recording techniques has been confirmed by measurement of $^{22}Na^{+}$ fluxes in the presence of 0.1mM veratridine. In the present study we have utilized a subclone of the SK-N-SN, the SK-N-SHIN (referred to as SHIN) which exhibit a remarkable degree of morphological differentiation when grown with 1mM dibutyryl CAMP. Fully differentiated SHIN cells have resting membrane potentials in the range of 50-80mV, which are much higher than the resting potentials seen in undifferentiated SK-N-SH cells. The input resistance and time constants of the differentiated SHIN cells is also increased. Most importantly, action potentials could be recorded from these cells after one week in culture. The amplitude of the spike potentials was variable, and the timing of spike initiation depended on the extent of development of the local potential induced by outward current injections from the cell body. These findings indicate that the spike generating mechanism is present on the processes and is distal to the cell body. In fact spikes were often recorded in cells with processes longer than 100μ. The presence of an Na^{+} dependent depolarizing component of the action potential generating mechanism was verified by the use of TTX. The TTX blockade of the action potential implies the presence of Na^{+} channels in the cell membrane. In other experiments, this was further confirmed where the passive influx of $^{22}Na^{+}$ was stimulated by veratridine: Such a stimulation is a property of fully excitable cells. In summary, we suggest that extensive morphological differentiation of this human neuroblastoma is a necessary prerequisite of the acquisition or expression of Na^{+} channels and the development of electrical excitability.

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1678 MORPHOLOGICAL PROPERTIES OF THE DENDRITES AND AXONS OF DISSOCIATED RAT SYMPATHETIC NEURONS. Story C. Landis, Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Neurons dissociated from the superior cervical ganglia of newborn rats and grown in mass cultures extend processes which eventually form a complicated network. Within this network, as in other culture systems, it is difficult to distinguish axons from dendrites with the phase microscope or in random thin sections. This raises the question of whether neurons which differentiate in culture develop distinct axons and dendrites with properties similar to those in vivo. Single neurons were grown in microcultures, approximately 300 μm in diameter, with small numbers of heart cells, then serially thin-sectioned perpendicular to the substrate so that processes were cut in cross section and reconstructed. In this simpler system, it is possible to clearly distinguish axons and dendrites.

Most neurons possess one to six relatively thick processes identifiable as dendrites which stain heavily with silver. These processes are up to 50 μm long and taper from 5 μm in diameter near the soma to approximately 2 μm near the tip. They may branch and end in a blunt tip or in finger-like extensions. Each process contains a central core of neurofilaments surrounded by microtubules scattered in a relatively dense cytoplasm. Close to the soma, the processes contain rough endoplasmic reticulum and free polysomes, but more distally only smooth endoplasmic reticulum and mitochondria are present. These processes are post- but not presynaptic and in general resemble the dendrites of sympathetic neurons in vivo except for their lack of small catecholamine-containing vesicles.

Distal regions of the axons are easily distinguished from dendrites. They are smaller, 0.5-1 μm in diameter, except at varicosities or synapses. The axoplasm is electron-lucent and contains predominantly microtubules with some neurofiliaments. Smooth endoplasmic reticulum and mitochondria are present but less prominent than in dendrites. The axon arises from the soma, but it extends for some distance before it acquires the appearance of the distal axon. This proximal portion contains more neurofilaments, not arranged in a central core, and denser axoplasm than the distal axon. A typical initial segment is lacking. Axonal processes are presynaptic to both dendrites and the soma, but not to other axonal processes.

Thus dissociated sympathetic neurons in culture develop processes which are morphologically very similar to dendrites and axons of sympathetic neurons in vivo.

Supported by NINCDS grants NS 11576 and NS 02253 and the Northeast Chapter of the Mass. Heart Assoc.

1679 **THE EFFECTS OF BUPIVACAINE (MARCAINE) ON CULTURED SKELETAL MUSCLE CELLS.** **G. J. Markelonis*, T. H. Oh and E. C. B Hall-Craggs*. Dept. Anatomy, Univ. Maryland Sch. Med., Baltimore, MD 21201.**

Intramuscular injection of the local anesthetic Marcaine produces degeneration of skeletal muscle fibers followed by rapid regeneration (Hall-Craggs, Exp. Neurol. 43: 349, 1974). We have now investigated the effect of this drug on chick embryo skeletal muscle fibers. Serial observations were made of selected fields of cultures under the inverted microscope. When exposed to Marcaine (1×10^{-3}M) spontaneous contraction of fibers ceased immediately. Within 10-20 min fiber diameter had decreased, cross-striations disappeared, and longitudinal striations become more apparent. Over the same period blebs appeared on the surface of fibers, increased in size and finally become detached from the fiber. This finding was more pronounced in young myotubes. At 30-40 min nuclei rounded up and seemed to dissociate from the surrounding cytoplasm leaving a clear area. These changes were more frequently observed in peripherally located nuclei which on occasions were seen to be partially separated from the fiber. After 60-120 min all fibers showed gross structural disorganization and subsequently detached from the substrate. At a similar concentration the drug produced cytoplasmic vecuolation and the appearence of small blebs in mononucleate precursor cells and in fibroblasts. Cultures exposed to a lower concentration of Marcaine (5×10^{-4}M) showed loss of cross-striations in mature fibers and the appearence of blebs in young myotubes. However, withdrawal of the drug after 24 hrs was followed by regeneration. In conclusion, Marcaine causes degenerative changes in muscle fibers, young myotubes and mononucleated cells in vitro. The myotoxic effects of the drug are exhibited as changes in the morphology of the contractile apparatus and nuclei, and a reductien of fiber diameter associated with the extrusion of cytosol. Supported by Grants from NIH (NS13296) and NSF (BNS76-15370).

1680 NGF ALTERS SPECIFIC PROTEIN SYNTHESIS IN RAT PC12 CELLS. Jeffrey C. McGuire* and Lloyd A. Greene*. (SPON: Nicholas T. Zervas). Dept. of Neuropathology, Harvard Medical School and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, MA. 02115.

PC12 cells are a clonal line derived from a rat adrenal medullary pheochromocytoma. PC12 cells respond to ngm levels of 2.5 S Nerve Growth Factor (NGF) by stopping cell division and acquiring neuronal properties, e.g., electrical excitability, long processes and small clear vesicles. We have investigated the protein composition of PC12 cells by SDS polyacrylamide gradient gel electrophoresis. Of the 60 bands consistently resolved on SDS gels, 5 are found in significantly increased amounts in NGF-treated cells (MWs ca. 290,000; 232,000; 75,000; 65,000; 21,000) and 1 is found in decreased amounts after NGF treatment (MW 24,000). Such changes in composition occur within 2 days after addition of NGF and take place before cessation of cell division and the beginning of process outgrowth. Similar results are seen on stained gels and on autoradiographs of gels labeled by growth of cells in medium containing labeled amino acids. The effect of NGF on synthesis of these proteins is reversible within 2 days after removal of NGF from pretreated cells. The RNA synthesis inhibitor camptothecin, when added with NGF, blocks both morphological and protein changes caused by NGF without affecting the relative levels of other proteins. The protein changes caused by NGF do not appear to be related to cessation of cell division since they are not caused by treatment with 1 μM cytosine arabinoside. The changes do not require morphological differentiation since they are not blocked by 0.1 μM colchicine, which prevents process outgrowth in NGF. We conclude that NGF stimulates the synthesis of specific proteins at the transcriptional level. This effect precedes, and may be required for, NGF-induced process outgrowth by PC12 cells. Supported by grants from NIH, Sloan Foundation, National Foundation-March of Dimes.

1681 DIFFERENTIAL EFFECTS OF DIMETHYL SULFOXIDE AND ELEVATED TEMPERATURE ON MYOBLAST FUSION. A. Miranda, G. Nette*, S. Khan*, K. Brockbank* and M. Schonberg*. Depts. of Pathology and Neurology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Dimethyl sulfoxide (DMSO) induces differentiation in culture neuroblastoma and erythroleukemic cells. Application of this agent (2%) to L8 cells, a rat-derived transformed myogenic line (Richler and Yaffe, 1970) for 48h or longer causes a modulation of their growth pattern from a randomly oriented, multilayered cell sheet to a regular whorled monolayer of flattened elongated fibroblast-like cells, which, even upon removal of DMSO fail to fuse and form myotubes. The treated cells which adhere more firmly to the substrate exhibit elevated endogenous cyclic AMP levels. The cells remain viable, but the population doubling time, mitotic index and pulse-label thymidine index decline. During DMSO treatment and subsequent removal there is no significant rise in creatine phosphokinase (CPK) levels.

Cultivating L8 myoblasts for 72 h at 41°C induces massive fusion of these cells with coincident rise of CPK (3-fold or higher). In identical cultures grown at 37°C myoblast fusion is less extensive, and occurs later (120h). When the cells are cultivated at cloning density for 72h, 80% or more of the cell colonies exhibit moderate to extensive fusion at 41°C, whereas only 15% of the colonies grown at 37°C show some degree of fusion. Myoblast cultures treated with DMSO as indicated above fail to fuse on exposure to elevated temperature. In this system DMSO does not promote myogenic differentiation but elevated temperature accelerates it.

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1682 SURVIVAL OF CHICK CILIARY GANGLION NEURONS IN DISSOCIATED CELL CULTURE WITH MUSCLE AND THE FORMATION OF NERVE-MUSCLE SYNAPSES. Rae Nishi and Darwin Berg. Dept. Biol., UCSD, La Jolla, CA 92093.

Chick ciliary ganglion (CG) neurons are attractive for studying neuronal survival and synapse formation. Between days 8 and 14 *in ovo*, cell death reduces the number of neurons/CG from 6500 to 3200(1). The remaining neurons innervate iris striated muscle and choroid layer smooth muscle. *In vitro* studies have shown that intact ganglia can innervate skeletal muscle(2), and some dissociated neurons can survive for several days when grown with heart-conditioned medium(3). We have found that dissociated CG neurons survive in large numbers for weeks when grown in culture with skeletal muscle and that many of the neurons form functional nerve-muscle synapses.

Ganglia from 8 day old embryos were dissociated by enzymic and mechanical means to yield 1.2×10^4 neuronal + non-neuronal cells/ganglion. When added to muscle cultures *ca.* 50% of the total cells (90% of the possible neurons) attached to the substratum, extended processes, and took on a characteristic neuronal morphology within 24 hrs. The number of such cells (5600±200 per original ganglion, mean±SEM) remained constant in culture over a 5 week period. Intracellular stimulation of the cells elicited conventional action potentials. In contrast, only 20% of the cells plated with conditioned medium on polyornithine- or collagen-coated dishes in the absence of muscle were able to extend processes in the first 24 hours, and less then 5% remained at 6 days.

Many muscle fibers in CG-muscle cultures repeatedly twitched spontaneously. The presence of functional nerve-muscle synapses was demonstrated by stimulating neurons with intracellular or extracellular microelectrodes while using an intracellular electrode to record evoked postsynaptic potentials (PSPs) in muscle. Synaptic transmission was detected as early as 20 hours after adding CG neurons to muscle cultures. At 1 week about 70% of the neurons tested with intracellular stimulation evoked PSPs in nearby muscle fibers contacted by the neurons (18/25 neurons, 6 cultures). Evoked PSPs were blocked by 0.4 μg/ml α-bungarotoxin and reversibly blocked by 50 μg/ml d-tubocurarine.

Thus CG neurons can express highly differentiated functions in culture with skeletal muscle. More neurons survive in culture than predicted from *in vivo* cell counts, and no decline in neuronal survival is observed with culture age. These observations raise the possibility that the muscle cells have rescued neurons in culture that would have undergone cell death *in vivo*. (Supp. by USPHS Grant #12601-01, Musc. Dyst. Assoc., & Amer. Heart Assoc.)

(1) Landmesser, L. and Pilar, G., J. Physiol. 241: 737 (1974).
(2) Betz, W., J. Physiol. 254: 63 (1976).
(3) Helfand et al., Dev. Biol. 50: 541 (1976).

1683 MOUSE MYOGENIC CELL LINES G7 AND G8. John H. Peacock, Daphne Rush* and Mark Noble*. Dept. Neurol., Stanford Univ. Sch. Med., Stanford CA 94305.

Fetal mouse myoblasts from primary muscle cell cultures have a capability for extended proliferation in culture when conditions are such to discourage myoblast fusion and subsequent differentiation into myotubes. For culture, muscle was dissected from hindlimbs of Swiss Webster mouse fetuses of 17 and 18 days gestational age. Factors contributing to continued cell division include subculturing at low cell densities and use of plastic not collagen-coated growth surfaces. 6 floating cell passages are possible and occasionally more than 10 trypsin dissociated passages can be obtained. One culture was colony cloned after several passages and clones G7 and G8 obtained. These clones differ from each other with respect to chromosome number, growth characteristics, morphology, acetylcholine receptor levels, capability for neuromuscular synapse formation by dissociated spinal cord neurons, and tumorigenesis in host mice.

Clone G7 has a modal number of 40 chromosomes and a generation time of about 19 hours. Myoblasts fuse poorly, despite increased calcium concentrations up to 10mM in growth medium. Both the large mononucleated cells and the thin myotubes containing few nuclei have acetylcholine (ACh) sensitivity and can contract with electrically elicited action potentials. Occasional spontaneous contractions occur. In comparison to G7, G8 has a modal chromosome number of about 67 and a generation time of about 25 hours. Differentiated G8 cultures form large multinucleated myotubes which contract spontaneously.

G8 cultures have 8-12 fold greater specific binding of ^{125}I-α-bungarotoxin (fmol/mg prot) to ACh receptors than G7. Both autoradiography and ACh iontophoretic mapping of G8 myotubes reveal nonuniform receptor localization on many cells which have focal ares ($<2\mu M^2$) of high receptor density and ACh sensitivites up to 8000 mv/nC and background sensitivities of about 500 mv/nC or lower. Other cells show uniformly high sensitivites of about 1000 mv/nC. Of interest, ACh responses in G8 exhibit little or no desensitization to ACh.

Postsynaptic potentials (blocked by 10^{-7}M d-tubocurare) occur in about 30% of G8 myotubes cocultured with spinal cord neurons and less than 1% of similarly cultured G7 myotubes. Thus G8 can be innervated as readily as primary mouse myotubes (about 20%).

G8 cells formed no tumors (0/7 mice) after subcutaneous innoculation into host mice but G7 cells were tumorigenic (6/7 mice). Histology of G7 tumors show the two cell types found in culture.

The different properties of these cell lines offer several comparative features for investigation of myogenesis and nerve-muscle interaction. (Supported by NIH grant NS12151 and NSF grant GB43526 to J.H.P.)

1684 GLIAL PROPERTIES OF A HUMAN CLONAL CELL LINE. B. Rothman*, R.L. Suddith*, L. Eng, D. Obert*, E. Tiffany*, K. Werrbach-Perez*, J.R. Perez-Polo and B. Haber (Spons. J.S. Kittridge), The Marine Biomedical Institute, and Dept. of Human Biological Chem. and Genetics, and Dept. of Neurology. The University of Texas Medical Branch, Galveston, Texas 77550, and Dept. of Pathology, V.A. Hospital, Palo Alto, California 94305.

The BT-510 is one of a number of clonal cell lines derived from a primary culture of a human brain tumor, metastatic from the lung. In culture, the BT-510 cells display an astrocytic-like morphology, similar to the rat C_6 astrocytoma. Chromosomal analysis of the BT-510 confirms that it is a human aneuploid cell line with a mean chromosomal number of 39. Aneuploidy was established by standard Giemsa banding techniques. In order to establish the glial nature of this cell line, we measured the levels of the glial fibrillary acidic protein (GFA). The BT-510 cells grown to stationary phase contain approximately 16% of the GFA content present in the C_6 cells. We have previously shown that choline transport in a variety of glial cell lines is stimulated by lowering the Na^+ concentration in the incubation medium (Neurochem. Res. 1, 201, 1976). Choline transport in the BT-510 cells was similarly stimulated by the withdrawal of Na^+ from the media. These human cells were found to have 9.7 nM/mg. Prot/min of GPDH activity. Prolonged exposure of stationary BT-510 cells to cortisone did not significantly enhance the specific activity of GPDH in the cells; in contrast to the established cortisone induction of GPDH in C_6 cells, and confirmed in these experiments. We have further compared the effects of cortisone on the expression of Monoamine Oxidase (MAO) in the BT-510 cells and in the C_6 astrocytoma (ATTC-CL107). Concentrations of cortisone ($5x10^{-7}M$) which induce GPDH in C_6 cells also markedly increase MAO activity in these experiments; the increase is as great as three times control values. In contrast, the MAO activity in BT-510 cells is only slightly elevated by higher concentrations of the hormone ($5x10^{-5}M$). The BT-510 cells are similar to other glial lines tested in their susceptibility to the cytotoxic effects of 6-hydroxydopamine. In summary, this cell line appears to be a reasonable model in which to study some properties of human glia.

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1685 KINETICS OF SYNTHESIS AND RELEASE OF ACETYLCHOLINESTERASE MOLECULAR FORMS IN CHICK MUSCLE CULTURE. Richard L. Rotundo* and Douglas M. Fambrough. Carnegie Institution of Washington, Dept. of Embryology, Baltimore, Maryland 21210.

Four molecular weight forms of acetylcholinesterase (AChE) can be distinguished in normal chicken muscle by their sedimentation constants (Vigny, et al., FEBS Letters 69: 277, 1976). Of these, three can be found in chick muscle grown in culture: during the first 5 days only the 6.5S and 11S forms are observed; however, after this time small amounts of a lower molecular weight form become apparent. We find part of the muscle AChE associated with the outer portion of the cell membrane. In addition to the three forms of AChE present in the myotubes, a considerable amount of enzyme is released by the cells in the culture medium (Wilson, et al., Develop. Biol. 33: 285, 1973). We find that its sedimentation constant is intermediate between those of the two major cellular forms, and that during velocity sedimentation the enzyme moves as a broad band, suggesting molecular heterogeneity. This released form of the enzyme is precipitable by Concanavalin A, indicating it to be a glycoprotein.

Using a sensitive radiometric assay for acetylcholinesterase and culture medium pretreated with diisopropylfluorophosphate (DFP) to inhibit serum and embryo extract esterases we have studied the kinetics of synthesis and release of AChE by chick embryo muscle cells in culture. Following the addition of fresh DFP treated medium there is a rapid accumulation of AChE in the medium. This accumulation is temperature dependent, is inhibited by puromycin (10 µg/ml) and cycloheximide (100 µg/ml), and is blocked by 10^{-3} M dinitrophenol or 10^{-5} M carbonyl cyanide-m-chloro phenyl hydrazone. Experiments involving inhibition of protein synthesis indicate an intracellular pool of AChE molecules equivalent to the amount released during a three hour period. The glycoprotein nature of the AChE molecule, its association with the outer membrane of the cells, the size of the intracellular pool, and the effects of various metabolic inhibitors on the release of the enzyme in culture are consistent with models of release involving either secretion or sloughing off of the membrane. The temporal and biochemical similarities between the release of AChE on the one hand, and the incorporation of the acetylcholine receptor molecule into the muscle cell membrane on the other, suggest that the two processes are metabolically related.

1686 ANTI-OLIGODENDROCYTE SERUM DEMYELINATES CULTURED CNS TISSUE. T. Saida*, O. Abramsky*, D.H. Silberberg, D. Pleasure, R.P.Lisak and M. Manning*. Dept. Neurol., U. of Pa., Phila., Pa. 19104.

The cytotoxic effect of anti-oligodendrocyte serum (AOS) was studied using cultured cerebellum. Oligodendrocytes were isolated from calf white matter using the method described by Poduslo and Norton. Purified oligodendrocytes were essentially devoid of attached myelin when studied with the electron microscope. Biochemical studies support the purity of the isolated cell preparation (Pleasure et al, Trans. Am. Soc. Neurochem., 1977). Using indirect immunofluorescence technique, AOS stained oligodendrocyte surface components in brain sections as well as in suspension. The antisera also reacted weakly with myelin but not with other brain cells (Abramsky et al, Neurology 4, 342, 1977).

7 New Zealand rabbits were injected with oligodendrocytes with complete Freund's adjuvant (FA) and reinoculated monthly with complete or incomplete FA. Myelinated mouse cerebellum cultures were exposed to nutrient medium containing 30% AOS with 10% guinea pig serum as a source of complement. AOS from 4 rabbits, after the second and third inoculations, produced demyelinative changes several hours after application to cultures. Oligodendroglia showed degeneration of cytoplasmic organellae and pyknotic changes, and splitting and destruction of myelin were seen, with electron microscopy. Axons remained intact. New formation of myelin was inhibited when mouse cerebellum cultures were continuously exposed to 5% AOS with 5% guinea pig serum, from the time of explantation. Both demyelination and myelination inhibition were heat-labile (complement dependent). The activities were removed by adsorption with oligodendrocytes but were only partially decreased by adsorption with myelin and not affected by adsorption with liver or kidney. Myelinated mouse dorsal root ganglian cultures showed no change after 5 days application of 50% AOS. Antigalactocerebroside and antiganglioside antibody levels were not elevated in the active sera, as determined by an agglutination test using liposomes containing the appropriate lipid hapten.

These findings suggest that antibodies directed to oligodendrocyte surface components, which appear to be distinct from antigalactocerebroside antibody, can destroy oligodendroglia and produce demyelination. Similar antibodies might be involved in the immunopathogenesis of demyelinating disorders.

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1687 DEXAMETHASONE-INDUCED MORPHOLOGICAL DIFFERENTIATION OF A MURINE C-1300 NEUROBLASTOMA CLONE. D. Sandquist*, T.H. Williams, S.K. Sahu*, S. Kataoka*. Dept. of Anatomy, University of Iowa, Iowa City, Iowa, 52242.

Previous work has shown that glucocorticoids have profound effects on developing neural crest tissue. This includes an increase in the number of SIF cells in superior cervical ganglion tissue *in vivo* and *in vitro* and in extra-adrenal chromaffin tissue, and an increase in phenylethanolamine-N-methyl transferase activity of the adrenal medulla and sympathetic ganglia (for review, see Chapter 15, SIF Cells, Fogarty Internat. Center Proc., No. 30). Dexamethasone, a potent synthetic glucocorticoid, was used to investigate differentiation responses in neuroblastoma, a malignant tumor of neural crest origin, grown in tissue culture.

NBP_2, a clone derived (by K. Prasad) from murine C-1300 neuroblastoma, was used. 50,000 cells were plated per 60 mm dish and treated the following day with dexamethasone 10 or 50 µg/ml (10 µg/ml = 0.025 mM). Controls were either untreated or treated with the solvent (ethyl alcohol). Drug and medium (F-12 with 10% GG-free newborn calf serum and antibiotics) were changed daily. 400 cells in separate areas were counted daily and scored as differentiated if extending a process twice the soma diameter. Six cultures were scored per treatment. Dexamethasone 50 µg/ml produced morphological differentiation in almost 65% of the population after four days of treatment. Dexamethasone 10 µg/ml produced differentiation in over 40%. Both treatments induced significantly more differentiation than untreated and solvent-treated controls, which did not differ significantly from each other. Dexamethasone also inhibited population growth. By 2 days post-treatment there was complete inhibition with dexamethasone 50 µg/ml. When the drug was withdrawn after 4 days of treatment, recovery of growth was slow, requiring 4 days to double cell numbers. By phase contrast microscopy, dexamethasone-treated cells appeared to have larger somata and more numerous perinuclear granules.

In conclusion, dexamethasone has a dose-dependent effect on morphological differentiation and growth inhibition of this neuroblastoma clone. This is in addition to the drug's effect on catecholamine fluorescence, previously reported (Anat. Rec. 187:704, 1977). Additional experimental results relating to the mechanism of action of dexamethasone will be discussed.

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1688 CELLULAR IMMUNE RESPONSES TO MYELIN BASIC PROTEIN IN MULTIPLE SCLEROSIS AND STROKE. William Sheremata, Susan Colby-Germinario* and Edwin H. Eylar*, Department of Neurology, McGill University Montreal, Quebec, Canada.

In vitro evidence of cellular immune responses to myelin basic protein (BP) has been reported to correlate with clinical activity of multiple sclerosis. This suggests an *in vivo* role for such sensitization in pathogenesis. In the present study lymphoblastic transformation (LBT) and the Thor-Rocklin MIF assay were used simultaneously to detect cellular immune responses to BP. Eleven acute multiple sclerosis patients were matched with 11 stroke patients for age, sex and time after onset of neurological deficit and the results compared with 24 control subjects. Seven of 10 MIF results in MS patients were significantly different ($p < 0.01$) as compared with normals (Mean index = 76 + 21.7). One of five patients suffering first stroke gave a positive assay (MI = 127 + 35) and all patients with repeat strokes (MI = 69 + 8). Three of eleven multiple sclerosis patients (MI = 1.83 + 1.4), but no primary stroke patients (MI = 1.00 + 0.45), and only one of 4 repeat stroke patients (MI = 1.51 + 1.6) showed a significant LBT response. Of the patients with other illnesses one patient each with Guillain-Barre, asthma, andsepticemia gave positive MIF responses, but only one with septicemia gave an LBT response.

An initial episode of brain damage did not result in the earlier appearance of the cellular immune response to myelin basic protein, but repeated injury may do so. Further studies will be required to illucidate the time of appearance and magnitude of this response in young stroke patients and further evaluate the significance of this response in multiple sclerosis and in stroke.

1689 γ-GLUTAMYL TRANSPEPTIDASE ACTIVITY IN CULTURED NEURONS, GLIA, ENDOTHELIA AND ISOLATED BRAIN CAPILLARIES. D.H. Shine*, R.L. Suddith*, H. Eisenberg and B. Haber, The Marine Biomedical Institute, Depts. of Human Biological Chemistry and Genetics, Neurology and Division of Neurosurgery, University of Texas Medical Branch, Galveston, Texas 77550.

γ-glutamyl transpeptidase (γ-GTP) (2.3.2.1) is an enzyme that catalyzes the transfer of the γ-glutamyl moiety of glutathione to amino acid or peptide acceptors. This enzyme may function in part as an amino acid transport system in tissues such as liver, kidney, pancreas and choroid plexus, where its presence has been demonstrated. We have measured the activity of γ-GTP in a preparation of mouse brain microcapillaries, using a colorimeteric assay and an artificial substrate, γ-L-glutamyl-p-nitroanilide. The capillary preparations have significant levels of both γ-GTP and Na^+/K^+ ATPase activities. At present, it is not clear whether the γ-GTP activity of brain capillaries is localized in the endothelial cells, or reflects a contribution of astrocytic end feet present in such bulk isolated capillary fractions. We have therefore measured the γ-GTP activity in several clonal lines of neuronal and glial origin. The SH-IN, a subclone of the SK-N-SH human neuroblastoma and the rat neuronal line (B-50) have low, but measurable levels of γ-GTP. In contrast, subclones of the rat C_6 astrocytoma ($C_6$1A, $C_6$2B), the rat glial line (B-104) and a human line (BT510) of probable glial origin developed in this laboratory all have γ-GTP activities with specific activites minimally tenfold higher than those seen in the neuronal lines tested todate. In preliminary experiments on the subcellular localization of γ-GTP in the C_6 cells, maximal specific activities of this enzyme are found in a fraction defined as microsomal. All the cell lines tested exhibit maximal activity in the stationary phase of the growth cycle, and both neurons and glia have roughly a similar Km (10mM). Human endothelial cells in primary culture, obtained via collagenase digestion of postpartum umbilical veins do not have any γ-GTP activity, as measured by the enzymatic assay. The high specific activity of γ-GTP in several lines of cultured glia coupled with the absence of such enzymatic activity in endothelial cells of peripheral origin suggests that the γ-GTP activity of brain microcapillary preparations reflects contamination by astrocytic end feet. Alternately, the presence of γ-GTP in endothelial cells of CNS origin may reflect the inductive effects of glial and/or neuronal interactions with capillary endothelium in the CNS.

Supported by Welch Grant H-504, and PHS Grants NS 11255 and NS 11354. The B-lines, C_6 astrocytoma subclones, and SK-N-SH cells were kindly supplied by Dr. D. Schubert, J. de Vellis and J. Biedler respectively.

1690 ELECTROPHYSIOLOGICAL RESPONSES OF SINGLE SMOOTH MUSCLE CELLS TO ACETYLCHOLINE, NORADRENALINE, AND HISTAMINE. C. Nelson Sinback* and William Shain. AFRRI, Bethesda, Maryland 20014.

Smooth muscle cells of a continuous line derived from human oviduct retain the same electrical properties as smooth muscle cells in vivo including action potentials and electrotonic current spread among cells (Sinback and Shain, Neuroscience 1976). Using dissociated cultures we describe the distribution of acetylcholine, noradrenaline, and histamine receptors on individual smooth muscle cells--something which cannot be studied in vivo due to current spread among cells.

We located receptors on 138 cells using (1) single iontophoretic electrodes containing histamine, acetylcholine, or noradrenaline, or (2) using two iontophoretic electrodes: one containing histamine and the other containing acetylcholine or noradrenaline while recording intracellularly with 1 or 2 microelectrodes. Single smooth muscle cells isolated from one another have receptors for acetylcholine, noradrenaline, and histamine. However, each cell did not have all three receptors. Virtually all cells tested (89%) contained histamine receptors, about half the cells tested (54%) contained acetylcholine receptors, and about half the cells tested (46%) contained noradrenaline receptors.

Acetylcholine and histamine each elicited one of three electrical responses from single cells. For instance, in 68 cells, histamine elicited hyperpolarizing responses in 37 cells (54%), depolarizing responses in 13 cells (19%), and biphasic hyperpolarizing-depolarizing responses in 9 cells (13%). All three responses were due to increased ion flow, desensitized, and were blocked by antagonists. Histamine receptors did not increase the flow of the same ions in every cell since hyperpolarizing responses and depolarizing responses had different reversal potentials.

The cell dimensions (20μ x >100μ) allowed mapping of receptor distribution on the membrane by iontophoresing a constant charge of histamine or acetylcholine at different positions. Receptors were concentrated on cell membranes, causing localized regions of high sensitivity to histamine and acetylcholine. Responses elicited in regions of high receptor density were >10 times the amplitude of responses in surrounding areas of low receptor density. Also, single cells segregated receptors causing hyperpolarizing responses and receptors causing depolarizing responses. This segregation of hyperpolarizing and depolarizing receptors was responsible for biphasic responses.

In conclusion, these results establish that the contributions of single smooth muscle cells to neural and hormonal responses of smooth muscle tissue, which cannot be studied in vivo, can be studied using dissociated cultures of single smooth muscle cells.

1691 NEURAL ACTIVITY IN AGGREGATING CULTURES OF FETAL RAT CNS. Carl E. Stafstrom, Daniel Johnston, David Brus, and J. R. Sheppard. Dight Institute of Human Genetics and Dept. Neurol., U. Minn., Minneapolis, MN 55455.

Extracellular recordings from fetal rat brain reaggregate cultures indicate that this system may prove valuable in the study of CNS development. The reaggregates are prepared by forcing brain tissue through a series of nylon sieves, which generates a suspension of single cells. When the cells are transferred to a flask containing tissue culture medium on a gyratory shaker (78 rpm), they reassociate into uniform 1 mm spheres and reorganize themselves within these spheres.

Using standard extracellular recording techniques, predominantly negative-going spontaneous neural activity is observed as a microelectrode (6-10 MΩ) contacts the reaggregate surface. Activity of maximum amplitude is observed just below the surface, and activity often reverses sign with deeper electrode penetration. The activity sometimes exhibits rhythmic behavior (1-3 Hz). A developmental sequence appears to be followed; amplitudes average 0.3 mV the first day in vitro, which increases to 1 mV on the fourth and subsequent days. This method of amplitude averaging allows quantitative investigation of the ionic and pharmacological mechanisms underlying the electrical activity, by comparing discharges of reaggregates from a control medium with discharges from reaggregates incubated in various experimental solutions. Preliminary results indicate that tetrodotoxin or a lowered Na^+ concentration increases the average amplitude of the activity, whereas Mg^{++} or Mn^{++} (a known Ca^{++} antagonist) appear to decrease the activity. Each of these agents exhibits a characteristic dose-response curve. Activity amplitude is also affected by several hormones and other pharmacological agents.

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1692 CHANGE IN THE DISTRIBUTION OF ACETYLCHOLINE RECEPTORS AFTER INNERVATION OF MUSCLE FIBRES IN TISSUE CULTURE. Willem F. Stevens*, Dick W. Slaaf*, Jacob Hooisma*, Tom Magchielse* and E. Meeter*(SPON: B. Kulig). Med. Biol. Lab. TNO, Rijswijk, The Netherlands.

The distribution of the acetylcholine (ACh) sensitivity along muscle cells is considered to be indicative for the maturation of the neuromuscular junction in vivo. In innervated and reinnervated muscle fibres the ACh sensitivity of the motor endplate region is much higher than that of the non-endplate region. Denervated and foetal muscle fibres are highly sensitive to ACh over their entire membrane.

In tissue culture an anomalous situation has been reported. The membrane of muscle cells, grown for about 10 days in culture , contains spots of hypersensitivity to ACh, so-called "hot-spots". These hot-spots are formed in the absence of innervating neural tissue but disappear in the mature muscle fibres. In our hands cultured non-innervated muscle fibres develop no hot-spots, which is a great advantage for the study of the effect of innervation on receptor density.

Chick leg muscle cells were grown in bicarbonate buffered MEM medium with 15% horse serum and 5% embryo extract, pH 7.3, 320 mosm. Sensitivity to ACh was measured by iontophoretic application of ACh, the density of the receptors by autoradiography using ^{125}I-α bungarotoxin (gift Z. Vogel, Rehovot, Israel).

With the electrophoretic method 81.000 μm^2 of membrane of non-innervated myotubes was tested with very small steps along the membrane. In an individual fibre the ACh sensitivity never varied more than a factor of 3.6, whereas the average variation was 1.5 x. Abrupt spatial differences in sensitivity were never found. These results were confirmed by autoradiography.

In myotubes cultured together with either chick ciliary ganglia or mouse spinal cord explants ACh sensitivity was measured in muscle cells which showed endplate potentials. The overall ACh sensitivity was found similar to that found in non-innervated myotubes. In the vicinity of possible endings of neurites, however, areas were found where the sensitivity was abruptly higher than that of the background. In such areas the mean ratio in ACh sensitivity over background was 10.2 (range 4.1-36.4)in myotubes innervated by the ciliary ganglion and 5.8 (range 4.3-8.1) if the cells were innervated by spinal cord explants. Autoradiography showed that many fibres in a small circular zone around the explant contained (several) clusters of ACh receptors, whereas myotubes further away from the explant had hardly any cluster on its surface.

These observations are consistent with earlier observations about the distribution of innervated myotubes around an explanted ciliary ganglion (Hooisma et al., Brain Res.85, 79, 1975) and with the region in which a spinal cord explant exerts its trophic influence (Hooisma et al., in preparation).

1693 FINE STRUCTURE OF MECHANICALLY DISSOCIATED RAT CNS AGGREGATING CELL CULTURES. B. D. Trapp*, P. Honegger*, H. deF. Webster and E. Richelson (SPON: M. R. Murray). NINCDS, NIH, Bethesda, MD 20014 and Dept. Pharmacology, Mayo Foundation, Rochester, MN 55455.

To supplement earlier biochemical studies (Honegger and Richelson, 1976) the morphology of mechanically dissociated fetal (15-16 d gestation) rat brain was investigated at 4, 19, 26 and 40 days in vitro. The aggregates were spherical in shape and ranged in diameter from 337μ ± 78μ (mean ± SD) at 4 days in vitro to 430μ ± 53μ at 40 days in vitro. At 4 days most cells were undifferentiated; some macrophages and ependymal cells were also present. At 4 and 19 days, mitotic figures were apparent; the neuropil was loosely packed and contained growth cones. Synaptic contacts and initiation of myelin formation were found in 19 day cell aggregates. At 26 days in vitro most cells could be identified as neurons, astrocytes or oligodendrocytes. The neuropil was tightly packed and synaptic complexes and compact myelin sheaths were present. At 40 days in vitro degenerative changes were observed in central regions of the aggregates. An increase in filaments within astrocytic processes and a dramatic decrease in the number of synapses was found at 40 days. In summary, cells within aggregating cultures differentiate prior to 26 days in vitro. Synapses and myelin sheaths are found, as suggested by biochemical data on similar cultures. Our results provide additional evidence that these aggregating cell cultures are useful for correlative biochemical, pharmacological and morphological studies of CNS development.

1694 EARLY CHEMICAL EVENTS INDUCED BY GLIA MATURATION FACTOR. David E. Turriff, Shuang S. Troy*, John Prunskis*, Taiji Kato* and Ramon Lim. Brain Research Institute, University of Chicago, Chicago, IL 60637.

The protein, Glia Maturation Factor (GMF), induces the morphological and biochemical differentiation of cultured glioblasts to mature astrocytes. Previously, we have reported that enhancement of DNA synthesis occurs at twelve hours after exposure to GMF (Lim et al., Science, 195, 195, 1977). This increase remains even with our most highly purified sample. However, inhibition of DNA synthesis with cytosine arabinoside does not inhibit the response of glioblasts to GMF. Enucleated glioblasts do not differentiate after GMF stimulation, however, indicating that a complex relationship exists between the nucleus and the differentiation capacity of the cells. We have detected increases in guanosine cyclic monophosphoric acid (cGMP) as early as one hour after stimulation with GMF. Attempts to correlate the cGMP increase with increased DNA synthesis and mitosis have failed since the cyclic nucleotide analogues, 8-bromo cyclic GMP and dibutyryl cyclic GMP, do not enhance glioblast cell division or DNA synthesis. Furthermore, bromo- or dibutyryl cyclic GMP neither cause glioblast differentiation by themselves nor enhance GMF-stimulated maturation. Consequently, although cGMP increases are the earliest detectable response to GMF, no role has yet been found for this cyclic nucleotide in the maturation process. (USPHS Grants No. 09228, CA-19266, NS-07376 and NS-05017).

1695 DISSOCIATED CELL CULTURE OF CHICK CILIARY GANGLION: SURVIVAL AND DEVELOPMENT WITH AND WITHOUT TARGET TISSUES. Jeremy B. Tuttle.* (SPON: Guillermo Pilar). Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268.

The avian ciliary ganglion and ciliary nerve-iris are preparations used extensively in studies of neural development. This usefulness might be extended if methods for long-term culture of these tissues were available. We report here that ciliary ganglion cells can be maintained in dissociated cell culture for extended periods (7-8 wks), and that the survival of some cells is not entirely dependent upon the presence of the natural target tissue (iris muscle).

Ganglion cells removed at different embryonic stages, dissociated by several techniques, and cultured in different media formulations, display wide variations in survival. In the least successful cases, cell death was immediately apparent, and only non-neural cells remained at 24-48 hrs in culture. Other regimes and formulations allowed survival of some neurons for 4-10 da. However, the most successful combination supported ganglion cell survival and development for about two months. Optimum age for isolation was found to be 7 da. in ovo (St. 31-32), survival was best when gentle trypsinization (0.08% trypsin) with mechanical disruption was used, and the medium was a modified Eagle's MEM, with 10% (v/v) heat-inactivated horse serum and 4-5% CEE_{50}. Supplemental NGF was not included in the medium. The substrate used was not critical for survival, as poly-D-lysine and poly-L-ornithine (PORN)-coated tissue culture dishes and collagen-coated surfaces all supported some cell survival. Collagen surfaces promoted non-neural cell growth, while PORN-coated ones did not. "Microcultures", or low-density "island" cultures on small spots of substrate were also prepared and axonal "connections" could form between adjacent "islands".

The developmental status of ganglion cells in culture was examined using several criteria. Ganglion cells could generate essentially normal action potentials soon after explantation, and retained this excitability for several weeks. If co-cultured with chick iris or leg myoblasts, the ganglion cells could rapidly form junctions with the muscle tissues. Cultures of ganglion cells also synthesize ^{3}H-acetylcholine from ^{3}H-choline, which suggests that the synthetic pathway for this neurotransmitter is present in culture. These results, and those of ultrastructural studies, indicate that prolonged culture of dissociated parasympathetic neurons is possible, even in the absence of exogenous NGF, and that long-term interactions between parasympathetic neurons, their target tissues, and other cell types may now be examined in cell culture. Supported by NIH-NS 10338 and NS 5382-01 and the Univ. of Connecticut Research Foundation.

TROPHIC FUNCTIONS

1696 LOCALIZATION OF NERVE GROWTH FACTOR IN THE VERTEBRATE BRAIN. Larry I. Benowitz and Victor E. Shashoua, McLean Hospital, Mailman Res. Ctr., Dept. Biol. Chem., Harvard Medical School, Belmont, MA 02178.

Nerve growth factor (NGF), a trophic protein required for the development and maintenance of certain ganglionic neurons, is synthesized in vitro by C6 glioma (Murphy et al., J. Cell. Biol., in press), and by glia derived from dorsal root and sympathetic ganglia (Varon, Exp. Neurol. 48, 75, 1975). A central occurrence of the protein has traditionally been doubted, although homogenized neuraxes of fish, as well as those of embryonic mouse and chick, have been shown to exert NGF-like activity in culture (Winick & Greenberg, Pediatrics 35, 225, 1965). To determine whether NGF is actually localized in specific elements of the vertebrate CNS, brain sections from goldfish, mouse and chick were reacted first with monospecific rabbit antisera against NGF (courtesy M. Young and R.A. Murphy), then with a marker antibody, goat anti-rabbit IgG conjugated with fluorescein.

An extensive plexus of fibers immunologically reactive with anti-NGF serum was found to be present throughout all levels of the goldfish brain. The fibers were plentiful in the ependymal zone from the spinal cord up through the forebrain, and in certain other sites (e.g., fiber and outermost layers of the vagal lobe) that are rich in glial processes. This labeling pattern partially corresponds with that of the glial fibrillary acidic protein (GFA-- derived from fibrous astrocytes: Dahl and Bignami, Brain Res. 61, 279, 1973), and is also similar to the distributional pattern of fibers containing β, a 32,000 MW soluble protein whose turnover rate is altered by conditioning in the goldfish (Shashoua, PNAS 74, 1743, 1977; Benowitz & Shashoua, Brain Res., in press). However, unlike the β protein, NGF is not found in cell bodies. In the mouse and chick, a much less extensive fiber plexus was stained with the anti-NGF serum, confined primarily to external and periventricular surfaces of the brainstem. These results indicate that while a protein immunologically similar to NGF may be associated with processes of brain cells, presumably non-neuronal, in all 3 vertebrate classes, it is particularly rich in the primitive ependymal zone of the fish. (Supported by The Medical Foundation, Inc. and The Scottish Rite Schizophrenia Research Program.)

1697 PHYSIOLOGICAL EVIDENCE FOR TROPHIC EFFECTS: IXth NERVE SECTION CAUSES RAPID FAILURE OF TASTE RESPONSES. Daniel Berland*, Joyce Chu*, Lee B. Jones*, Jon Kaliszewski*, William Lawler*, and Bruce Oakley. Div. Biol. Sci., Univ. Mich., Ann Arbor, MI 48109.

Neural innervation is a necessary factor for the maintenance and regeneration of mammalian taste buds. The mechanisms of such neurotrophic dependencies have remained obscure, although chemical agents have long been suspected. We have developed a physiological assay system for the neurotrophic effects of taste axons using the IXth nerve of the Mongolian gerbil. From the intact IXth nerve summated multi-unit discharges to chemical stimulation can be recorded. These responses are stable for at least 14 hours. The relative responsiveness to different chemicals is similar, but not identical, to that of the rat IXth nerve. NH_4Cl is the most effective of the 15 chemicals tested. If the gerbil's IXth nerve is transected, the taste responses will rapidly decline to 0-25% of the initial summated response. The responses do not always decline in parallel for compounds representing the four basic tastes (0.5M sucrose, 0.03M HCl, 0.3M NaCl, and 0.01M quinine hydrochloride). This loss of evoked activity does not result from degeneration of the axons with subsequent failure to propagate impulses because: (a) compound action potentials can still be elicited by electrical stimulation of a cut IXth nerve that has become quiescent to chemical stimulation of the tongue, and (b) there is no difference in the rate of response decline monitored simultaneously from two sites on the nerve, one near and one far from the cut end. Efferent fibers are unnecessary for maintaining the physiological responses. When the IXth nerve is *cut proximal to the petrosal ganglion normal taste* responses can be recorded from the intact distal portion of the nerve 5, 7, 11, 13, and 30 days later. The time of taste response failure (1-4 hours) is a linear function of the length of the distal IXth nerve stump attached to the tongue. This suggests that the taste axons may maintain taste buds by transported trophic chemicals. Accordingly we tested procedures, such as local nerve cooling, designed to block axoplasmic transport in the uncut nerve. A nearly complete loss of taste responses was observed 1-4 hours after treatment.

We conclude 1) that chemical interactions, rather than impulse activity, are responsible for the trophic effects upon taste buds and 2) that the short-term physiological function of taste buds is maintained by the continued anterograde or retrograde transport of chemical agents along sensory axons.

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1698 NEUROTROPHIC REGULATION OF PROTEIN SYNTHESIS IN REGENERATING NEWT FORELIMBS: AN IN VITRO ASSAY OF PUTATIVE NEUROTROPHIC FACTORS. A.H.F. Choo*, D.M. Logan* and M.P. Rathbone. Dept. of Biol., York University, Toronto, and MRC Group in Developmental Neurobiology, Dept. of Neurosciences, McMaster Univer. Med. Centre, Hamilton, Ontario, Canada L8S 4J9.

Regeneration of amputated amphibian limbs requires the presence of nerves which regulate the rates of DNA, RNA, and protein synthesis in the regenerates. The infusion of nerve or brain homogenates into denervated limb regenerates in vivo mimics this neurotrophic effect and has been used as a bioassay for factors with putative neurotrophic activity (Singer, M. et al., J. Exp. Zool. (1976) 196:131-150). Because this is a technically difficult assay and variability in the rates of limb regeneration produces variable results, we have developed an alternative simple, reproducible in vitro assay for testing neurotrophic activity. Newt forelimbs are amputated just proximal to the elbow. The resulting blastemas are amputated after 18-20 days. Second blastemas form after 6-7 days and are explanted into amphibian culture medium containing radioactive amino acids. The second blastemas are synchronized in developmental stage and have similar rates of macromolecular synthesis. After denervation in vivo or culture in vitro the rate of protein synthesis in blastemas increases transiently, but subsequently falls and remains below the values observed for protein synthesis in blastemas of innervated limbs. Addition of nerve extracts to culture medium of denervated synchronized blastemas prevents these changes and maintains normal protein synthesis. Blastema proteins are separated using two dimensional isoelectric point focusing-polyacrylamide gel electrophoresis. There are differences in the incorporation of radioactive amino acids into various proteins of innervated and denervated blastemas. The incorporation of radioactivity into proteins of blastemas cultured with nerve extracts is similar to that of blastemas denervated in vivo. Thus the effects of nerve extracts in vitro are similar to those of nerves in vivo. Using this assay with synchronized blastemas cultured for 10 hours, we have demonstrated that the neurotrophic activity in chick brain extract is heat labile and destroyed by proteases. It has a molecular weight of <20,000 daltons. Although the activity is restricted to nerve tissue it is not species specific and is found in neural tissue of both newts and chickens.

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1699 A TROPHIC FACTOR FROM A PRESYNAPTIC NEURON INCREASES ACETYCHOLINE RECEPTOR AGGREGATION ON MYOTUBES. C.N. Christian*, M. Daniels, H. Sugiyama*, Z. Vogel* and P.G. Nelson, NIH, Bethesda, Md. 20014 and Weizmann Institute, Rehovot, Israel.

During synaptogenesis between NG108-15 hybrid cells and embryonic mouse muscle cells cultured in vitro, hybrid cells probably seek out or produce areas of high acetylcholine (ACh) sensitivity on myotubes (Christian, et al., Science 196:995).

We studied the distribution of ACh receptors on mouse myotubes by indirect immunoperoxidase staining of bound α-bungarotoxin (Daniels and Vogel, Nature 254:339). One third of the cultured mouse myotubes exhibited local aggregates of ACh receptors, with an average diameter of 10 μm. Hybrid cells were added to muscle cultures and cocultured for one week in Dulbecco's Modified Eagle Medium plus 5% Horse Serum and 1 mM dibutyryl cyclic AMP (dbCAMP), a medium in which these two cell types form functional cholinergic synapses. Mouse myotubes cocultured with hybrid cells showed a 3 fold increase in the number of ACh receptor aggregates per myotube (A/M) when compared to myotubes in cultures without hybrid cells. In 4 separate experiments comprising 7 cocultures, the increase in A/M ranged from 1.8 to 4.4 fold. The increase in A/M did not require dbCAMP.

Medium from hybrid cell myotube cocultures, diluted by 50% with fresh medium, produced a 2.2 fold increase in A/M when added to muscle cultures. Medium without serum from hybrid cell cultures produced a 3.4 fold increase in A/M in muscle cultures. The effect was not produced by media from human diploid fibroblasts or HeLa cells, nor by medium plus 1 mM ACh.

These striking changes in A/M do not result from a modulation of a number of ACh receptors. Hybrid cell medium did not change the number of myotube receptors by more than 10%, as shown by measurements of ^{125}I-α-bungarotoxin binding to control and hybrid cell medium treated muscle cultures. Hybrid cell medium produced a 3.1 fold A/M increase within 21 hours in the presence of 100 ug/ml of cycloheximide.

These observations suggest that NG108-15 hybrid cells produce a factor which aggregates ACh receptors present on mouse myotubes. The factor may cause the alignment of cholinergic presynaptic release sites with postsynaptic aggregations of acetylcholine receptors, and may play a more general role in neuron muscle recognition.

1700 NEURAL CONTROL OF CHLORIDE CONDUCTANCE IN RAT EDL MUSCLE. T.E. DeCoursey*, S.H. Bryant and S.G. Younkin (SPON: R.J. Lipicky). Dept. Pharmacol. and Cell Biophys., Univ. of Cincinnati Coll. of Med., Cincinnati, OH 45267.

It was previously reported that exposure of nerve to colchicine resulted in a reduction of resting membrane chloride conductance (G_{Cl}) in rat fast (EDL) muscle (Camerino and Bryant: J. Neurobiol. 7:221, 1976), as has been observed following denervation of mammalian skeletal muscle. Neuromuscular transmission was not directly measured in that study, and Westgaard's finding that direct stimulation of denervated rat soleus can return total membrane conductance (G_m) to normal levels (J. Physiol. 251:683, 1975) raises the possibility that the reported reduction of G_{Cl} after colchicine treatment was the result of a possibly significant loss of muscle activity.

We exposed the sciatic nerve to cotton soaked in 30 or 60 mM colchicine in 0.9% saline, for 45 minutes. Five to seven days later, indirect twitch and tetanic tensions were measured *in vivo* in EDL muscles from treated and contralateral control sides. Muscle cable parameters were then measured using the two-electrode square pulse technique, in normal and (methylsulphate-substituted) chloride-free physiological solutions. Resting membrane resistance (R_m) in chloride-free solution was assumed equal to the reciprocal of potassium conductance (G_K). G_m was assumed equal to $1/R_m$ in normal solution, and equal to G_K+G_{Cl}.

Indirect twitch and tetanic tensions were about 40% lower in experimental than contralateral EDL muscles in 4 rats ($p<0.01$). This reduction presumably reflects a substantial loss of impulse transmission to at least some fibers. In this group of animals, mean (±SE) G_{Cl} was 18% lower in treated than control fibers (2081±223 and 2552±309 μmho/cm^2). In a second group of 4 rats, twitch and tetanic tensions were not significantly different from control. In these animals G_{Cl} was 29% lower ($p<0.01$) in fibers from the treated side (2184±49 and 3095±160 μmho/cm^2). G_K was not significantly different from control in either group; combined mean control and treated were 165±18 and 198±48 μmho/cm^2.

If the reduction of G_{Cl} after acute exposure of nerve to colchicine were due to a loss of neuromuscular activity, G_{Cl} would presumably be reduced to a greater extent in the group of rats exhibiting significant loss of impulse transmission than in the group with normal response to indirect stimulation. Since this did not occur, we conclude that G_{Cl} was reduced in this study by an action of colchicine on nerve which is not related to a change in muscle activity.

1701 NEUROTROPHIC PROTEIN(S): ROLE OF AXONAL TRANSPORT IN THEIR INTERCELLULAR MOVEMENT. Hugo L. Fernandez, Myron Duell*, Thomas G. White* and Barry Festoff, Neurobiology Research Lab, V.A. Hospital, Kansas City, MO 64128 and University of Kansas Medical Center.

An extract of rat sciatic nerve, termed soluble nerve protein (SNP) was previously shown to: a) cause weakness and neuromuscular fatigue in SNP-immunized sheep; and, b) produce antibodies against three polypeptide components of SNP (1). It was further demonstrated that SNP enhanced growth and differentiation of chick embryo muscle cells in culture (2). The suggestion of these studies was that some component(s) of SNP served a "trophic" function and that the syndrome elicited in the sheep may have been an "autoimmune" response to some similar endogenous factor present in the sheep. Since axoplasmic transport (AT) has been shown to be an important mediator in the "trophic" regulation of skeletal muscle (3), the present work was undertaken in an effort to determine whether SNP is conveyed by A.T.

SDS-PAGE electrophoresis, immunoelectrophoresis, and double immunodiffusion of SNP, prepared from 1 cm sections of rat sciatic nerve and spinal cord, showed that: the major component of SNP is an acidic 65,000 daltons protein; this component in both sciatic nerve and spinal cord cross-reacts selectively with sheep anti-SNP antibody; and, it is distinct from tubulin in terms of MW and immunological specificity.

Quantitative radial immunodiffusion of SNP indicated that 10-20 days after ligating the sciatic nerves this major SNP protein component accumulates both proximal and distal to the ligatures. Accordingly, this SNP component would appear to be conveyed by retrograde, as well as, orthograde A.T. It must be noted, however, that 6 hr after intraspinal injection of 25 Ci ^{3}H-leucine into the L5 ventral roots, labeled SNP was not detected around the sciatic nerve ligatures. In turn, significant amounts of labeled SNP were found close to the injection site. It follows that if the major component of SNP is transported, it must do so at a slow rate of A.T. Alternatively, what we have termed SNP might reflect Schwann cell cytoplasm. Current experiments are designed to further define the origin of this SNP protein.

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1702 BIOCHEMICAL STUDY OF THE FISH VISUAL SYSTEM DURING NEURONAL REGENERATION. Dana Giulian* and David Cowburn* (SPON: B. S. McEwen). The Rockefeller Univ., and Cornell Univ. Med. Coll. New York, N.Y. 10021.

In order to investigate biochemical events involved in the regeneration of transected retinal ganglion axons of the goldfish, a separation of cell types and identification of major proteins of the visual system was carried out. Microdissection has yielded a unique preparation of isolated ganglion cells that was free of glial elements as shown by light microscopy and transmission and scanning electron microscopy. Patterns of polypeptides studied by polyacrylamide gel electrophoresis in sodium dodecyl sulfate indicated that the ganglion cells and optic nerve fibers have a similar composition that contrast with other cellular layers of the retina or of the optic tectum. Actin and tubulin were major proteins of the ganglion dissection while myelin proteins were a significant component of the optic fibers. Studies of protein synthesis and axonal transport in normal tissue and, then in the regenerating system, was carried out by intraocular or intratectal injection of, or in vitro incubation with, labelled precursors.

The ganglion dissection was the only retinal layer to show increased ^{3}H-L-leucine incorporation during regrowth with a peak of synthesis around 30 days after transection. In addition to altered production and transport of major protein components, electrophoretic gel patterns showed the appearance of several minor polypeptides, one (about 150,000 daltons) in the regenerating ganglia by day 10 and another (~400,000 daltons) in the optic fibers by day 30. Events specific to the tissues and to the time course during regeneration have been identified by studying these profiles of synthesis. This provides ground work for the study of putative regulatory factors controlling specificity during regrowth.

1703 DOES NEUROTROPHIC MATERIAL CONTROL SYNAPSE FORMATION IN THE ADULT RAT BRAIN? Dan Goldowitz* and Carl W. Cotman. Dept. Psychobiol., Univ. Calif., Irvine, CA 92717.

Diamond and co-workers have obtained evidence from studies on the salamander hind-limb that collateral nerve growth occurs in normal nerves when a neighboring nerve is either sectioned or blocked from transporting material. These results suggest that a transported "negative neurotrophic substance" may regulate nerve growth in peripheral nerves. It was our purpose in these studies to determine if connections in the mammalian brain follow similar influences.

The molecular layer of the dentate gyrus was analyzed for possible changes in its synaptic density following applications of colchicine to the fimbria. From previous ultrastructural work it is known that in the weeks following a lesion of either the entorhinal or commissural afferents to the dentate gyrus there is a reappearance of synapses which restores the synaptic density of the neuropil to near-normal levels. We wanted to determine if, by the inhibition of axonal transport in the commissural fibers, we could induce synapse formation without the need of a lesion.

Colchicine applied to the fimbria inhibited axonal transport of proteins to the treated hippocampus and also interferred with the transport of AChE from the septum. Electron microscopic analysis of the fimbria and molecular layer of the dentate gyrus did not reveal the presence of degenerating fibers or boutons at 4, 11, or 60 days after treatment. Moreover electrophysiological analysis showed that colchicine application did not noticeably change the response of the commissural system, whose fibers course through the colchicine treated area. Over time, transport of radio-labeled proteins was restored to normal. At 60 days after colchicine treatment the density of synapses in the dentate molecular layer was surveyed by electron microscopy. There was a 20% increase in the number of synapses/unit area in the commissural region. These results suggest that colchicine treatment can induce a hyperinnervation of the dentate. Our finding supports the notion that a "neurotrophic substance" may regulate synapse formation in the mammalian CNS. (Supported by NIMH grant MH 19691 and NIMH Pre-doctoral fellowship MH 05308).

1704 NEURONAL STIMULATION OF [^{3}H]THYMIDINE INCORPORATION BY NON-NEURONAL CELLS. G.R. Hanson* and L.M. Partlow. Dept. Pharmacol., Univ. Utah Col. Med., Salt Lake City, UT 84132.

Stimulation of [^{3}H]thymidine incorporation in non-neuronal (glial) cultures by intact neurons was demonstrated by McCarthy and Partlow (Brain Res. (1976) 114:415) using highly purified and recombined primary cultures of neuronal and non-neuronal cells isolated from sympathetic ganglia of 11-day chick embryos (Brain Res. (1976) 114:391). The results presented herein further characterize this neuronal-glial interaction. First, the dose-effect relationship between the number of neurons added and degree of stimulation of glial [^{3}H]thymidine incorporation was found to be dose-dependent and biphasic. The dose-effect curve first plateaued at a neuron-to-glial cell ratio of 1:5 (~4-fold stimulation) but then increased further and reached a second plateau at a ratio of 1.2:1 (~ 50-fold stimulation). Second, the time course of neuronal stimulation of [^{3}H]thymidine incorporation by non-neuronal cells was examined. Neurons initially plated with the glia stimulated non-neuronal cell proliferation throughout the 72 hour period studied. In addition, neurons added to pure non-neuronal cultures after 48 hours in vitro stimulated [^{3}H]thymidine incorporation. Third, embryonic neurons stimulated [^{3}H]thymidine incorporation by slowly proliferating mature glia. Thus, addition of embryonic sympathetic neurons to cultures of sympathetic non-neuronal cells from 14-day posthatch chickens caused a 2.5-fold increase in [^{3}H]thymidine incorporation. Fourth, CNS and peripheral neurons stimulated both CNS and peripheral non-neuronal cells. Both optic lobe and sympathetic neurons from 7-and 12-day chick embryos, respectively, increased [^{3}H]thymidine incorporation in embryonic glial cultures from 12-day dorsal root ganglia, 12-day sympathetic ganglia, and 7-day cerebral cortex. In summary, neuronal stimulation of [^{3}H]thymidine incorporation by non-neuronal cells occurs in both central and peripheral portions of the nervous system, and in mature as well as embryonic glia. The stimulatory effect is dependent on the neuron-to-glial cell ratio and can increase non-neuronal proliferation by as much as 50-fold. (Supported by USPHS grants DE-05054 and NS-12812 and by a grant from the Epilepsy Foundation of America.)

1705 CENTRAL CONTROL OF NEURONAL NUMBER IN THE LATERAL MOTOR COLUMN OF CHICK EMBRYOS: AN EXPERIMENTAL EVALUATION. Randolph B. Malloy and C. H. Narayanan. Dept. Anat., L.S.U. Med. Ctr., New Orleans, La. 70119.

Peripheral control of cell number within a neuronal population has been shown in several investigations. There has been little attention directed to the possibility of afferent input in terms of its relationship to the control of cell numbers at cord levels. Three series of experiments were, therefore, done to determine if sensory input was in any way important in the regulation of cell numbers in the L.M.C. of the chick embryo at leg levels. In the first series of experiments, the neural crest was removed from lumbosacral cord levels by microcautery. In the subsequent experiments, a gap was placed just rostral to the lumbosacral cord and finally a combination gap and neural crest removal was accomplished. The embryos in control and the three experimental groups were sacrificed at 6 days, 12 days, and 18 days of incubation and the tissue was processed via routine histological procedures and stained using Orange G and Hematoxylin. Cell counts were accomplished on the lateral motor column (L.M.C.) of the lumbosacral cord in the four animals of each group. Analysis of L.M.C. cell counts at 6 days indicated no significant differences among experimental and control groups. At 12 days, however, the neural crest and neural crest plus gap groups had significantly reduced cell numbers when compared to gap and control groups. Results in the 18 day groups corresponded to the 12 day findings. These results indicate that central influences from afferent spinal cord sources do have an affect on the maintenance of L.M.C. cells of the chick embryo.

1706 TWO WAYS THAT AN ELECTRICAL CONNECTION IS RE-ESTABLISHED IN THE LEECH. Kenneth J. Muller and Salvatore Carbonetto* (SPON: D. Olton). Dept. of Embryology, Carnegie Institution of Washington, Baltimore, MD. 21210.

Precise regeneration of an electrical synapse between particular interneurons in the medicinal leech was traced physiologically and morphologically with intracellular recording and injection of horseradish peroxidase (HRP). HRP is particularly useful because it is visible both in light and electron microscopes and it does not fill neurons that are electrically coupled to the injected cell. The normal synapse between S-cell interneurons lies in the connective midway between segmental ganglia, except near the head and tail of the animal, and when the connective is crushed near one ganglion, the axon of only one S-cell is severed. In the ensuing days the injured cell regenerates, but its uninjured "target" cell in the next ganglion does not grow. The severed distal segment of the regenerating neuron continues for weeks to conduct impulses and remains connected to the uninjured target S-cell. After the sprouts of the regenerating neuron cross the crush, one or a few branches find the surviving distal segment and grow along it toward the region of normal synapse. In some cases there is particularly rapid restoration of electrical coupling and subsequent transmission between S-cells (within 2 or 3 weeks). In these instances the regenerating neuron has synapsed upon its severed distal segment, forming a basket of processes around the severed axon, but growth continues toward the point of normal synapse. Alternatively, the regenerating neuron's processes do not synapse until, after 3 or 4 weeks, they reach their normal target, where electrical junctions form and further growth ceases. Normal electrical transmission between the fine, regenerated fiber and the larger, target axon occurs in stages, including one-way transmission of impulses across the non-rectifying junction from the large fiber to the small but not vice versa. Within 2 or 3 months the regenerated neuron has grown to full caliber, the distal segment has degenerated and function is normal. These experiments indicate that the regenerating neuron is guided to its proper synaptic target by recognizing and following its severed distal segment. In some instances the distal segment itself becomes an intermediate, and in that sense imperfect, synaptic target.

1707

WITHDRAWN BY AUTHOR

1708 DIFFERENCES IN THE CATECHOLAMINE-SENSITIVE ADENYLATE CYCLASE AND β-RECEPTOR BINDING BETWEEN FAST-TWITCH AND SLOW-TWITCH SKELETAL MUSCLES OF RAT. K.L. Oliver*, N.B. Reddy* and W.K. Engel.
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One of the several differences that exist between mammalian fast-twitch (FT) and slow-twitch (ST) skeletal muscles is their differential response to β-adrenergic catecholamines (Catechols) (Bowman and Nott, Pharm. Rev. 21, 27, 1969). Since most of the effects of β-adrenergic Catechols on target tissues are believed to be mediated by activating adenylate cyclase (AC) through their interaction with β-receptors, we investigated the properties of AC and β-receptor binding in a sarcolemmal (SL) membrane preparation of FT (extensor digitorum longus) and ST (soleus) muscles of rat.

Basal and NaF-stimulated activities of SL-bound AC were about the same in both muscles. Activation of the AC by Catechols differed markedly in the two muscles. Isoproterenol (Isop), 10 μM, caused about 3-fold stimulation of ST SL AC while the FT SL AC was stimulated by only 60-80% over their respective basal activities. Similar pattern was obtained with epinephrine (Epi) and norepinephrine (NEpi). Phenylephrine (10 μM), an α-adrenergic agonist, showed no effect on SL AC of either ST or FT muscle. The order of potency of Catechols in activating AC was, however, similar in both ST and FT muscles and found to be Isop > Epi > NEpi. The EC_{50} values for Isop, Epi and NEpi were 0.1, 0.8 and 20 μM respectively in both ST and FT muscles. In both the muscles, the (-)isomers of Isop and Epi were at least two orders of magnitude more potent, on molar basis, than their corresponding (+)isomers. The augmentation of AC caused by Isop (10 μM) in SL of both muscles was completely abolished by β-blocking agents, propranolol and alprenolol. The (-)isomers of these β-blockers were more potent inhibitors than were their corresponding (+)isomers. The IC_{50}s for (-)isomers of propranolol (20 nM) and alprenolol (30 nM) were similar for the FT and ST muscles.

The β-receptor density in SL of ST and FT muscles was determined by following the binding of ^{3}H-dihydroalprenolol (DHA), a potent β-blocker. The specific ^{3}H-DHA binding (per mg SL protein) was 40-60% greater in FT than in ST muscle, which is in contrast to the activation of AC by Catechols in these two muscles.

The results suggest that: a) the level of Catechols-stimulated AC is not indicative of the β-receptor density, and b) the AC and β-receptor are independent entities and their extent of coupling is differentially regulated in FT and ST muscles, perhaps by yet-unidentified "trophic influence(s)" of nerves innervating each muscle. (N.B.R. supported by an MDAA Research Fellowship).

1709 NEUROTROPHIC FACTOR (NTF) EFFECT ON CHICK MUSCLE CELL GROWTH, DIFFERENTIATION, AND MAINTENANCE *IN VITRO*. Heinz Popiela*(SPON: J. E. Heavner). Dept. Biochem. University of Washington, Seattle, Wa. 98195.

An aqueous extract from adult chicken sciatic-ischiatic-peroneal nerves (NTF) was tested for growth promoting activity on dissociated embryonic chicken muscle cells and for maintenance activity on differentiated myotubes *in vitro*. Growth promoting activity of NTF was compared with embryo extract (EE) and other tissue extracts. The results indicate NTF to substitute for EE and to superiorly promote muscle cell growth. NTF maintains differentiated myotubes in a viable state and prevents or greatly reduces the characteristic degeneration of them as observed in its absence. After ischiatic-peroneal nerve ligation for 3 days, extractable NTF activity is reduced three fold from nerves distal to the ligation. NTF supports muscle cell growth only when concomitantly present with horse serum; NTF by itself does not sustain growth. When extracts from various embryonic tissues are compared to whole embryo extract no significant differences in activity are found. Abundant growth promoting activity extracted from embryonic brains is present at an extremely low concentration in brains of young adult or adult chickens. The results are interpreted as supporting the hypothesis that all embryonic cells produce a NTF-like substance(s) whereas in the adult certain nerve cells only retain the capacity to produce it while other cells in the adult have drastically reduced NTF-like substance(s) production (Singer, Quart. Rev. Biol. 27:169, 1952)

1710 NEURONAL AND GLIAL INTERACTIONS IN TISSUE CULTURE. Barry H. Smith, Maureen A. Ward*, Theodore Liszczak* and Paul K. Kornblith*.
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Neuronal and glial cell lines and conditioned media derived therefrom have been used to study molecular interactions regulating cell mitosis, growth, metabolic and maturational control. Normal human brain cell explants, as well as neuroblastoma and astrocytoma (Grades I-IV) cell lines have been utilized. All cells have been grown under constant culture conditions at 35°C with Ham's F-10 (Gibco) medium and with or without 10% fetal calf serum (Gibco). In an effort to confirm previous reports of glial-induced differentiation of neuroblastoma cell lines, medium "conditioned" for 72 hours in the presence of various human astrocytoma lines was added to neuroblastoma cultures. No consistent effect of such media was observed under our culture conditions for periods of 24 hours to 1 week. However, preliminary results suggest that neuroblastoma cells themselves may differentially inhibit the growth of certain astrocytoma cell lines, suggesting support for the idea of neuronal control of glial cell mitosis. Cell-cell contact appears to be necessary for the effect, but the involvement of a diffusable factor has not been ruled out. Finally, cyclic nucleotides and various other agents, including transmitters and hormones, are being examined for their effect on neuronal and glial mitosis and growth control. Dibutyl c-AMP alters human astrocytoma cell behavior in culture in a temporally limited fashion, dependent on the cell line. The quantitative and molecular mechanisms of such effects are under study.

1711 FAILURE OF PRODUCTS FROM DEGENERATING NERVE FIBERS TO INFLUENCE THE ELECTROPHYSIOLOGICAL PROPERTIES OF NORMAL FAST AND SLOW MUSCLE FIBERS. T. N. Tiedt*[1], E. X. Albuquerque[1] and L. Guth[2].
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As a result of numerous physiological and biochemical studies using a variety of experimental approaches the conclusion has been reached that peripheral nerve fibers release a trophic substance which maintains certain properties of the muscle membrane. An alternative explanation has been suggested that products of the degenerating nerve release substances which are responsible for inducing the denervation changes in muscle membranes. As evidence in support of the latter interpretation, Cangiano & Lutzemberger (Science, 196, 542, 1977) recently reported that the innervated muscle fibers of partially denervated muscles exhibited at the neuromuscular junction a resistance to the action of tetrodotoxin (TTX) similar to that of the denervated muscle fibers. To examine further this finding, partial denervation of the extensor digitorum longus muscle was accomplished by cutting spinal nerve L4 and of the soleus muscle by cutting L5. (1) In 16 rats examined 48-216 hours postoperatively, innervated surface fibers (i.e., fibers from which miniature endplate potentials (MEPPs) could be recorded), had membrane potentials similar to those of the control, unoperated side (-77±0.6 mV, extensor; -76±0.4 mV, soleus). Denervated fibers (from which MEPPs could not be recorded) had membrane potentials of -57±0.5 mV (extensor) and -58±0.4 mV (soleus). (2) Extrajunctional acetylcholine sensitivity (range 100 to 400 millivolts/nanocoulomb) was found along the entire surface only in those fibers which were depolarized and which did not exhibit MEPPs. (3) Extrajunctional TTX-resistant action potentials were recorded along the surface of the denervated (depolarized and without MEPPs) fibers, while the innervated fibers of the same muscles in general showed no resistance to this drug either extrajunctionally or at the neuromuscular junction. Rarely (<1%) partially TTX-resistant action potentials were found at the neuromuscular junction of innervated fibers of control as well as partially denervated muscles. This phenomenon, which has also been reported by Thesleff et al. (Acta Physiol. Scand., 91, 196, 1974), thus cannot be considered as a sign of denervation. These results do not support the hypothesis that products of nerve degeneration influence properties of innervated muscle fibers but do support the hypothesis of neurotrophic regulation of muscle. (Supported in part by U.S.P.H.S. Grants NS-12063 and NS-12847.)

1712 EFFECT OF CHRONIC β-BUNGAROTOXIN TREATMENT ON THE DISTRIBUTION OF ACETYLCHOLINE RECEPTORS IN THE RAT DIAPHRAGM.
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Within a few days after axotomy the denervated muscle undergoes changes in electrical properties including the appearance of extrajunctional sensitivity to acetylcholine (ACh). This spread of ACh sensitivity following denervation can be mimicked by chronic application of botulinum toxin or β-bungarotoxin (β-BuTX), toxins which act presynaptically to inhibit ACh release. The ability of these toxins to mimic denervation suggests that continuous ACh release is necessary and sufficient to maintain the electrical properties of the normally innervated muscle. However, β-BuTX also has phospholipase A (PLA) activity, and presynaptic endings treated with β-BuTX undergo morphological changes reminiscent of denervated nerve endings. Therefore, the ability of β-BuTX to mimic denervation may be related to its degenerative action and not to its pharmacological effects. We have endeavored to separate the morphological and pharmacological actions of β-BuTX with regard to its ability to mimic denervation effects in muscle. Rat phrenic nerve-diaphragm muscle preparations were chronically treated for 1 to 14 days by intrathoracic application of enzymatically active or inactive β-BuTX. Active β-BuTX had PLA activity, while inactive β-BuTX had been heat treated at high pH so that PLA activity was eliminated. After treatment in vivo, diaphragms were removed and assayed for distribution of ACh sensitivity by iontophoretic application of ACh and by ^{125}I-α-bungarotoxin binding. We found that chronic treatment with equivalent amounts of either active or inactive β-BuTX yields a significant enhancement of ^{125}I-α-bungarotoxin binding over the whole muscle surface compared to untreated diaphragms. The increase in binding was not as great as that in denervated muscle at the same time period, however. The binding results were corroborated by electrophysiological findings as well. Untreated muscles responded only to ACh iontophoresed onto endplate regions; muscles treated with either active or inactive β-BuTX had junctional and extrajunctional sensitivity to ACh. Our results support the hypothesis that ACh provides a "trophic" influence which maintains electrical properties characteristic of normally innervated muscles.

1713 EFFECT OF ELECTRICAL STIMULATION ON LEVELS OF ACETYLCHOLINESTERASE ACTIVITY IN DENERVATED DYSTROPHIC CHICKENS. Paul M. Weidoff Jr.* and Barry W. Wilson. (SPON: R.C. Carlsen). Dept. Avian Sciences, Univ. of California, Davis, CA 95616.

Electrical stimulation of denervated muscles of normal chickens partially prevented the increase in acetylcholinesterase (AChE) activity that normally occurs with denervation and reduced its activity once it had risen (Linkhart and Wilson, Exp. Neurol. 48:557, 1975). It was suggested that electrically evoked excitation or contraction were partially responsible for this effect. A defect in the regulation of AChE exists in muscles of chickens with hereditary muscular dystrophy in which high AChE activity is present in the sarcoplasm around the motor end plate and spectrophotometrically measured activity in muscle homogenates is 2-3 fold higher than in the normal. This study examined whether or not denervated dystrophic muscle could respond to electrical stimulation in a manner similar to denervated normal muscle.

Posterior latissimus dorsi muscles of normal and dystrophic chickens were denervated and implanted with stainless steel wires for continuous electrical stimulation in situ. Trains of square wave pulses at a frequency of .2/S were continued for 21 H/day for 7 days. Voltages were adjusted to give supramaximal twitches and ranged from 0.5 to 6 V. At the conclusion of the stimulation program, stimulated and contralateral control muscles were removed and compared histologically and biochemically. In normal muscle, stimulation prevented 58% and in dystrophic muscle 54% of the increase in AChE activity that normally accompanies denervation. In addition, in dystrophic muscle 52% of the increase in non-specific cholinesterase activity that normally accompanies denervation was prevented, while no effect was noted in normal muscle. Lactate dehydrogenase activity was unaffected by stimulation in both normal and dystrophic muscles after denervation. Polyacrylamide gel electrophoresis of muscle homogenates from stimulated animals revealed the same molecular forms of AChE that appear with denervation alone. Histochemical staining for AChE in muscle cross sections showed intense extrajunctional activity after stimulation of dystrophic denervated but not normal denervated muscles. The results suggest that factors in addition to muscular contraction and bioelectric activity are involved in modulation of AChE activity in denervated dystrophic muscle. (supported by NIH grants GM01934, NS10957, and the MDA).

1714 PREVENTION OF THE DENERVATION INDUCED DROP IN RMP IN ORGAN CULTURED RAT EDL. S. G. Younkin, L. H. Younkin*, R. S. Brett*, and B. Davey*. Dept. Pharmacology, Univ. of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

In agreement with earlier studies we have found that denervation of rat EDL muscle causes the muscle resting membrane potential (RMP) to drop from 78.5±0.5 to 61.7±1.1 mV in 2 days. When rat EDL muscles were removed (and therefore denervated) and cultured for 2 days in 10 ml of oxygenated (95% O_2-5% CO_2) Trowell's T8 medium to which EDL muscle extract had been added much of the decrease in RMP was prevented (RMP=71.4±0.4 mV). Extracts were prepared by homogenizing tissue in 4 ml of T8 medium at 4° C using a Potter-Elvehjem homogenizer. After addition of 4 ml of T8 medium the extract was centrifuged in the cold (4° C) at 2700XG for 10 minutes. Additional T8 medium was then added to the supernatant medium so that each ml of the final supplemented medium contained the extract of 10 mg of tissue. Extracts of sciatic nerve (68.5±0.9 mV), whole brain (71.3±1.1 mV), whole diaphragm (71.2±0.5 mV), nerve free diaphragm (71.0±0.5 mV) and 15% rat serum (71.8±0.8 mV) were also effective in preventing the decrease in RMP. Muscles did not usually survive well in medium supplemented with liver extract so RMP could not be measured. Spleen extracts could not be demonstrated to prevent the drop in RMP associated with denervation (RMP=64.0±0.9 mV). The effect of muscle extract was dose dependent. As the amount of muscle extracted into each ml of T8 was decreased from 10 mg/ml to 0.1 mg/ml the mean RMP after culturing for 2 days fell from 71.1±0.4 to 63.8±1.0 mV. Much of the activity in muscle extracts seems to be in macromolecules having a molecular weight between 100,000 and 300,000. When whole diaphragm extract is passed through a filter (Amicon, XM300) which retains molecules of greater than approximately 300,000 M.W. the filtrate retains activity (RMP=71.1±0.6). When this filtrate is passed through a filter which retains molecules of greater than approximately 100,000 M.W. (Amicon, XM100A) activity is lost in the filtrate (RMP=64.4±1.4) but can be recovered by resuspending the filter cake in T8 and using this medium to culture (RMP=73.4±1.0). We are currently examining the effect of extracts on the decrease in acetylcholinesterase and the increase in ACh sensitivity which are caused by denervation.

VESTIBULAR SYSTEM

1715 A COMPARISON OF HORIZONTAL AND VERTICAL VESTIBULOOCULAR REFLEXES OF THE RABBIT. N. H. Barmack. Neurological Sciences Institute, Good Samaritan Hosp. & Med. Cntr., Portland, OR 97209.

Recent anatomical and physiological studies have suggested that different areas of the brain control vertical and horizontal eye movements. The present report examines whether these anatomical differences are evident functionally in the horizontal and vertical eye movements evoked by vestibular stimulation in unanesthetized rabbits. Rabbits were placed in a servo-controlled, bi-axial, rate table with their heads at the intersection of the vertical and longitudinal axes. The table was sinusoidally oscillated (.001 - .8 Hz, ± 10 deg) about the vertical axis, evoking the horizontal vestibuloocular reflex (HVOR), and about the longitudinal axis, which because of the lateral placement of the eyes of rabbits, evokes a vertical vestibuloocular reflex (VVOR). Eye movements were measured in total darkness with an infra-red light projection technique. Measurements of gain and phase of eye movements with respect to head movements were made. The gain of the HVOR was low, .2 - .3, and there was a large phase lead, 95 - 130 deg, at a stimulus frequency of .01 Hz. The gain increased to a maximum, .6 - .9, and the phase lead decreased to 10 - 15 deg at .1 Hz. At higher stimulus frequencies (.5 Hz) the gain decreased to .4 - .6, and the phase lead decreased further to 0 - 5 deg. The VVOR was dramatically different. The gain was high, .8 - .95, and the phase lead was 0 deg at a stimulus frequency of .01 Hz. As the frequency was increased from .01 Hz to .05 Hz the gain decreased to .7 - .8 and the phase lead increased to 5 - 10 deg. With further increases in frequency, the gain again increased and phase lead increased until at .1 Hz - .8 Hz the gain and phase of the VVOR were identical to the gain and phase of the HVOR. The high gain of the VVOR at low stimulus frequencies suggests that the VVOR is evoked by the conjoint action of inputs from the semicircular canals and otoliths, and that the functional contribution of the otolithic input occurs primarily at low stimulus frequencies (below .05 Hz). The dynamics of the eye movements evoked in the HVOR and VVOR are also different. At stimulus frequencies above .05 Hz, compensatory horizontal eye movements are repeatedly interrupted by saccades before reaching an extreme position of gaze. The occurrence of these re-setting saccades is dependent on both eye position and eye velocity. Vertical eye movements travel to more extreme positions and attain higher velocities before they are interrupted by re-setting saccades. These data suggest that the VVOR is based on two classes of afferent information, and that eye movements evoked by the HVOR and VVOR are controlled by different central neural mechanisms. (Supported by N.I.H. grant EY-00848 and The Oregon Lions Sight Foundation).

1716 FUNCTIONAL ORGANIZATION OF THE MEDIAL VESTIBULAR NUCLEUS (MVN) IN THE SQUIRREL MONKEY. L.P. Bernstein*, J.M. Goldberg, and C. Fernandez* (SPON: P.R. Huttenlocher). University of Chicago, Chicago, Illinois, 60637.

Single units were studied in the MVN of barbiturate-anesthetized squirrel monkeys. The topographic distribution of ampullary inputs was determined by the location of units responding to stimulation in the plane of a single canal pair. One canal pair was studied in each animal. Electrode punctures were reconstructed and assigned to one of five equal rostro-caudal zones (1 the most rostral). Units were designated in terms of the canal ipsilateral to the side of recording. Horizontal canal units (HC) were most strongly represented in zones 1 and 2 while superior canal (SC) and posterior canal (PC) units were most frequently encountered in zones 3 and 4. No segregation of inputs in the coronal plane was observed. Roughly half of the units had Type I and half Type II responses (typing after Duensing and Schaefer).

In a separate series of animals the response of units to individual stimulation of all three canal pairs was studied. Of the units responding to rotation approximately 75% were related to 1 canal plane. The remainder had convergent inputs from 2 (or rarely all 3) canal planes. Plugging the canals ipsilateral or contralateral to the side of recording did not appreciably change the percentage of convergent units. Most units had a bilateral and reciprocal response to polarization of the round window. More than 2/3 of responsive units could be monosynaptically activated from the ipsilateral side. None of the units studied responded only to tilt. Units sensitive to somatic stimulation were rarely encountered.

The MVN differs from the superior vestibular nucleus (Abend, Brain Res., in press) in having a stronger HC input, a larger number of Type II units, and a greater incidence of neurons influenced by stimulation of more than 1 canal plane. Supported by NIH and NASA.

1717 POWER SPECTRAL ANALYSIS OF POSTURAL FORCE TRAJECTORIES IN PATIENTS WITH PERIPHERAL, EIGHTH NERVE AND POSTERIOR FOSSA LESIONS. F. Owen Black, Conrad Wall, III and Dennis P. O'Leary, Dept. of Otolaryngol., Div. of Vestibular Disorders, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA. 15213

A computerized posturography technique was developed in order to quantitatively study characteristics of the human vestibulospinal control system in normal and vestibular deficient humans (1). The characteristics of postural control as reflected in fluctuations in the amplitude of center-of-force polar vectors were used as a basis for comparison. Specific characteristics were estimated from the power spectral densities, such as energy estimates obtained by integrating power spectra. The characteristics of postural control in these three groups of patients were found to vary systematically as functions of frequency of movement required to control upright posture and/or the energy content of the oscillations required to control center-of-force postural trajectories.

1. Black, F.O., et al, The Vestibulo-Spinal Stability Test (VESST): Normal Limits. In press: Trans. Amer. Acad. Ophthal. and Otolaryn., 1977

1718 EFFECTS OF VESTIBULAR STIMULATION ON CEREBELLAR DENTATE NUCLEUS NEURONS IN RAT. Robert H. I. Blanks* and Victoria Chan-Palay (SPON: S. L. Palay). Harvard Med. Sch., Boston, MA 02115.

Intra- and extracellular potentials were recorded from dentate neurons in nembutalized rats following electrical stimulation of the ipsilateral and contralateral VIIIth nerve. Dentate neurons were identified antidromically following stimulation of their projection sites to the brachium conjunctivum (latency 0.5-0.7 msec), interstitial nucleus of Cajal and ventrolateral nucleus of the thalamus (latency 0.65-0.9 msec). Recording sites in the dentate were confirmed by marking them histologically with the dye Pontamine Sky Blue 6BX. Many dentate neurons displayed a short-latency spike activation following ipsilateral (1.2-4 msec) and/or contralateral (1.5-6 msec) vestibular nerve stimulation, whereas other neurons remained unaffected by stimulus strengths of up to 4-6 x N_1 threshold (threshold for monosynaptic activation of second order vestibular neurons). The early activation observed in most neurons was most often evoked from stimulation of the ipsilateral labyrinth, but in a number of these neurons activity was evoked following stimulation of the contralateral vestibular nerve. On the basis of their latency and resistance to barbiturate anesthesia, it is suggested that these potentials were conveyed by a pathway involving one or possibly two interposed synapses between each labyrinth and dentate neurons. Occasionally, this early activation of dentate neurons was followed by suppression of spontaneous activity lasting approximately 40 msec which, when recorded intracellularly, was shown to result from vestibular-evoked IPSPs. Presumably these IPSPs were mediated by Purkinje cells simultaneously activated by the vestibular nerve stimulation. Histological verification of recording sites revealed that vestibular evoked activity was most often obtained from the caudal, ventral aspects of the nucleus.

The present study provides electrophysiological evidence for a direct, excitatory vestibulo-dentate projection. Such a pathway has been demonstrated anatomically, by Carpenter et al.(1972) in monkey, and Chan-Palay (1977) in rat and monkey. The significance of these connections needs to be further elucidated. However, since the dentate plays an important role in the control of distal limb musculature, vestibulo-dentate connections may be related to hand-eye coordination and head movements.
(This work was supported in part by National Institutes of Health Research Grants NS 10536 and NS 03659.)

1719 ACTIVITY OF UNITS IN AND AROUND THE VESTIBULAR NUCLEI OF ALERT MONKEYS DURING VERTICAL SMOOTH EYE MOVEMENTS AND VESTIBULAR STIMULATION. M.C. Chubb,* A.F. Fuchs, and G.W. Johanson,* (SPON: H.D. Patton). Regional Primate Center and Dept. Physiology and Biophysics, Univ. of Washington, Seattle, WA.

Previous studies have tested the relation of unit activity in the vestibular nucleus to horizontal vestibular stimuli and smooth pursuit. Little attention has been given, however, to unit firing patterns during vestibular stimulation and smooth eye movements in the vertical plane.

Single unit activity was recorded in and around the vestibular nuclei of alert monkeys that were trained to track a button while undergoing sinusoidal angular acceleration. Unit activity was studied during three conditions: 1) while the monkey was oscillated and the target moved 180°out of phase with his head. 2) while the monkey suppressed his vestibulo-ocular reflex, and 3) while he made smooth pursuit eye movements in the absence of imposed chair movement. Units ranged from those modulated with vestibular stimulation and not modulated during smooth pursuit to those strongly related to smooth eye movements with no apparent vestibular sensitivity. Activity of many other units was strongly related to both vestibular stimuli and eye movements.

When unit modulation was tested in planes 45° from the sagittal plane, to preferentially excite one coplanar pair of vertical canals, units usually showed an increase in modulation in one of these 45° planes and a reduced modulation in the other.

In cases where the horizontal sensitivity also was tested, many units were strongly modulated with both vertical and horizontal eye movements and angular acceleration. These observations suggest that there is convergence of horizontal and vertical canal input.

In general, the unit firing patterns in relation to vertical eye movements and adequate vestibular stimulation resemble those reported for the horizontal plane.

(Supported by Grant EY00745 from NIH)

1720 THE ROLE OF THE FLOCCULUS IN THE VESTIBULO-OCULAR REFLEX OF THE CHINCHILLA. P. D. Daniels*, M. Hassul*, J. Winfield* and J. Kimm. Univ. of Washington, Seattle, WA 98112.

The dynamics of the vestibulo-ocular reflex (VOR) in the chinchilla produce compensatory eye movements equal in magnitude to and 180° out of phase with head movements only at frequencies of rotation greater than 0.2 Hz if no visual fixation point is present. At lower frequencies, in the absence of visual input (i.e., in the dark) eye movement amplitude is lower than head movement amplitude and the phase of eye position leads the phase of normalized head position (head position minus 180°) by up to 60°. With a visual fixation point present, eye movements are in phase with normalized head movements at all frequencies tested (0.03 to 1.0 Hz).

After bilateral flocculectomy, two changes in the VOR response are observed:

1) The phase lead of the VOR response in the dark for a given frequency below 0.2 Hz is greater in the flocculectomized animal than in the normal animal. This effect can be quantified by approximating the VOR as a first order system, a "leaky integrator", with a time constant of 3 sec. for the normal animal and a time constant of 2 sec. for the lesioned animal.

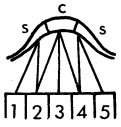
2) In contrast to normal animals, the presence of a visual fixation point does not modify the VOR response in lesioned animals. That is, the phase of the VOR of lesioned animals rotated with a visual target present is the same as the phase of the VOR of lesioned animals rotated in the dark.

A model of the role of the flocculus in the VOR of the chinchilla has been developed which accounts for these experimental observations. Since the output of the flocculus is known to impinge on brain stem structures involved in the VOR and it has been recently demonstrated that in addition to head velocity information the flocculus also receives eye movement and retinal information, we have incorporated the flocculus in our model as an important neural structure influencing visual-vestibular dynamics. The model shows that the flocculus can use eye velocity information to improve the time constant and can use retinal input to modify the low frequency phase response of the VOR.

The relation to this model of recent single unit data obtained in the chinchilla will be discussed in a separate presentation (Winfield, et al., this issue).

1721 PROJECTIONS INTO THE CRISTA'S RIDGE REGION OF THE HORIZONTAL AMPULLARY NERVE FIBERS IN THE GUITARFISH. Robert F. Dunn, Dept. of Otolaryngol. Div. of Vestibular Disorders, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA. 15213

The dynamic response characteristics of the nerve fibers comprising the horizontal ampullary nerve (HAN) of the ray, Rhinobatos productus have previously been shown to vary according to their position within the HAN (1,2). An analysis of serial cross-sections (1 μm, toluidine blue stained) was undertaken to determine the nerve fiber projection patterns by tracing individual nerve fibers from the HAN trunk (HANT) to the basilar lamina of the horizontal crista. The ridge region of the horizontal crista was defined as that area located between the ventral and dorsal plena semilunata. In cross-sections, the ridge of the horizontal crista may be divided into three portions, the anterior and posterior slopes (S) and the crest (C). The demarkation between the slope and the ridge approximates the mean angle of a line drawn tangent to the apical receptor surface of the slope compared to that at the crest. Whereas a projection pattern based upon division of the HANT into three equal parts was completed, the results presented in this communication are based upon a division of the HANT into five equal cross-sectional portions. The procedure entailed a color coding of the individual nerve fiber on each of the successive cross-sections with only those nerve fibers being included which extended from the HANT to penetrate the basilar lamina. Seventy-two nerve fibers satisfied this criterion, a sample which represents 5% of the mean number of fibers in the HAN (2).



The following projection patterns were considered consistent with the functional response data (2): HANT area 1 to slope; HANT 2 to slope and crest; HANT 3 to crest; HANT 4 to crest and slope, and; HANT 5 to slope, with the added constraint that the projections must be to the ipsilateral slope. Of the 72 nerve fibers followed, 86.1% (n=62) had these projection patterns; while 12.5% (n=9) projected from HANT 3 to the slopes; and 1.4% (n=1) crossed the midline by projecting from HANT 2 to the contralateral slope.

REFERENCES:
1. O'Leary, D.P., et al, Nature, 251: 225 (1974)
2. O'Leary, D.P., et al, J. Neurophysiol., 39: 631 (1976)

Supported by NIH research grants NS 09440 and 7 R01 NS 12494 from the National Institute of Neurologic and Communicative Disorders and Stroke.

1722 SINGLE UNITS RECORDED FROM THE VESTIBULAR NUCLEUS OF CATS WITH FREELY MOVING HEADS. James H. Fuller. Biol. Sci. Group, Univ. Connecticut, Storrs, CT 06268.

Single units were recorded from the vestibular nuclei of cats trained to make head movements between two targets displayed on an 80°-wide screen. Surgically implanted bolts fixed the cat's head to a shaft which allowed rotation about a vertical axis centered above the first-second cervical joint. The shaft could be rigidly fixed to the platform on which the animal was seated and the whole animal rotated about the same axis by a servo-controlled motor.

Units located in and near the medial vestibular nuclei showed a modulation of their firing rate when the animal was passively rotated with sinusoidal wave forms in darkness (head shaft fixed to platform); the peak firing rate was roughly in phase with peak velocity, suggesting a semicircular canal origin of the modulation. When a cat moved its head voluntarily or when the neck was deviated passively by externally fixing the animal's head and rotating the platform about the stationary head, there were slight, spurious changes in firing rate.

However, when impediments to voluntary head movement (inertia, elastic and static friction) were added, the units consistently fired when the animal moved its head in a direction opposite to that which elicited peak firing rates when the whole animal was passively rotated, i.e., opposite the presumed semicircular canal input. The modulation of firing during active movements was proportional to the position of the head if elastic resistance was added, or to the amount of torque produced (measured in the vertical and horizontal planes by strain gauges) if static friction was added. If the head was stabilized in space and the platform allowed to rotate freely, attempted head movements--now independent of semicircular canal stimulation--resulted in increased firing according to the amount of horizontal head torque produced, as on previous occasions in which the head moved against resistance.

It is proposed that these units, resembling those seen in monkey (Fuller and Miles, 1974, Soc. Neurosci.), receive an input representing the summed errors from an earlier stage; and their output reflects an error signal, representing any mismatch of intended and resulting head movements. The semicircular canal signal represents a final summed feedback signal for velocity control of head movement.

1723 EFFERENT VESTIBULAR SYSTEM IN THE SQUIRREL MONKEY. Jay M. Goldberg and César Fernández.* Depts. Pharm. Physiol. Sci. and Surgery (Otolaryngology), University of Chicago, Chicago, Ill. 60637

A retrograde transport technique was used to label efferent neurons in the neonatal squirrel monkey. Observations confirm those made in the cat (Gacek and Lyon; Warr). There are 300-400 ipsilateral efferent neurons and a comparable number of contralateral neurons. The distribution of labelled cells is similar on the two sides. The main efferent group consists of a relatively discrete column of cells located dorsal to the exiting facial nerve and interposed between the abducens and superior vestibular nuclei.

The efferent pathways were electrically stimulated, while afferent activity was monitored in Scarpa's ganglion. The uncrossed and crossed components could be activated either simultaneously or separately. In all cases, efferent stimulation resulted in excitation, reflected by an increase in background activity. Tetanic stimulation was required. Maximum effects were obtained at high shock rates (200-333/sec), but responses were also seen at more physiological rates (50/sec). Response dynamics were slow. Irregularly discharging afferents were characterized by responses 5-10 times larger than those of regularly discharging fibers. Three observations argue the specificity of the response. 1) The effects were abolished by intracranial vestibular-nerve section, but not by facial-nerve section or bilateral cervical sympathectomy. 2) The same electrode placement could result in excitation of vestibular afferents and inhibition of cochlear afferents, the latter effect presumably arising from stimulation of auditory efferents. 3) An intense afferent inhibition, which silenced afferent discharge, could also abolish the efferent response. Since afferent inhibition originates within the sensory epithelium, this suggests a similar locus for the efferent responses. There is a potential artifact not ruled out by our observations. The excitatory response, rather than being synaptically mediated, could possibly be the result of abnormal impulse traffic within the sensory epithelium which could influence afferent activity, say, by increasing extracellular K^+ levels. It is unclear, however, how such a mechanism could explain the differential responsiveness of regular and irregular afferents. On the other hand, were the responses synaptically mediated, the differences in the two classes of afferents could be accounted for by differences in the efferent innervation of Type-I and Type-II hair-cell systems.
(Supported by NIH and NASA)

1724 VISUAL FIXATION DURING HEAD SHAKE: ITS MODIFICATION BY LONG-TERM ADAPTATION TO VISION REVERSAL. A. Gonshor* and G. Melvill Jones (SPON: A.T. Tan). Aviation Medical Research Unit, McGill University, Montreal, Canada H3G 1Y6.

These experiments examine the effect of long-term plastic adaptation to vision reversal on the ability a) to visually track an oscillating target with head still and b) fixate a stationary target whilst oscillating the head about a vertical axis. All experiments were performed on a human subject at a time when he was fully adapted to prolonged (14-28 days) and maintained vision reversal and was consistently producing a reversed vestibulo-ocular reflex (VOR) in response to sinusoidal rotation at 0.17 Hz in the dark. dc EOG and a head-mounted electromagnetic search coil measured eye and head movement respectively. Head oscillation was voluntarily generated with a sinusoidally oscillating sound to "pace" the frequency of oscillation. The mean gain of ocular response (eye angle/head angle) was assessed from the peak to peak amplitudes of 10 consecutive cycles of eye and head rotation. Gains were assessed respective-ly as positive or negative according to whether the mean phase of eye movement was less than 70°, or greater than 110° shifted from that of target movement relative to the head. All tests were made in normal light on the fully adapted subject but with the reversing prisms temporarily removed.

No modification was ever seen in the frequency dependent limi-tation to the visual tracking of a moving target with head still. However, marked changes were found with the head oscillating relative to a stationary target. Instead of the normal moderate increase of ocular gain from about +0.9 to +1.0 over the frequency range 0.5 to 5.0 Hz, the following mean gains were found in the adapted subject: (-)0.5 at 0.5 Hz, (-)0.1 at 1.0 Hz, +0.2 at 2.0 Hz, +0.4 at 3.0 Hz and +0.5 at 5.0 Hz. These findings are surprising since one might have expected that at low frequencies visual tracking would have successfully predominated over the reversed (ie adapted) VOR, whilst at high frequencies the reversed VOR would have predominated since visual tracking then becomes ineffective. In reality we found the exact opposite of these anticipated results! The findings are however compatible with a lower frequency response in the adapted (multineuronal?) than the original (disynaptic?) VOR central pathways. It is concluded that the visual tracking capability per se is not modified by long-term adaptation to vision reversal, but that there is "more than meets the eye" in the resulting VOR modification.

Supported by Canadian MRC

1725 VESTIBULAR COMPENSATION: INFLUENCE OF SPINAL CORD ON SPONTANEOUS ACTIVITY OF VESTIBULAR NUCLEI. David W. Jensen. Dept. Neurosciences, Sch. Med., UCSD, La Jolla, CA

One of the most striking examples of plasticity of the nervous system is demonstrated by the disappearance of the overwhelming postural asymmetries that follow a unilateral labyrinthectomy in higher vertebrates ("vestibular compensation"). Spinal lesions or limb blockades are known to disrupt the compensatory symmetry ("decompensation"). A study was performed on the neural correlates of decompensation. Spinal preparations of guinea pigs which had compensated to a unilateral labyrinthectomy were monitored for the return of spinal reflex activity and then given a postbrachial spinal transection or lumbar plexus blockade with 2% Xyolocaine. No decompensation asymmetry was measurable in passive resistance to stretch or in forelimb extensor and neck EMG activity, which remained at low levels. Compensatory changes confined to the spinal cord are therefore unlikely to account for body postural decompensation asymmetry. Integrated multiunit activity in the vestibular nuclei (VN) of intact and compensated animals was tested for effects of cold blocks performed at spinal T-7. The animals were locally anesthetized and paralysed; temperature and heart activity, monitored continuously, remained steady throughout. For a given locus, the net multiunit VN activity either did not change, decreased, or increased in response to the T-7 cold block. Each type of effect was reversible, repeatable and consistent for a given preparation. Complex responses were also obtained, including single units in a population that behaved differently from the rest of that population. Fluctuating levels of spontaneity were commonly seen also. In the 31 loci studied that proved to be in the Descending VN (DVN) (14 loci in 5 intact, and 17 loci in 5 compensated animals), the mean effect in intact DVN was a 0.3% increase, while compensated DVN showed a mean 27.7% decrease ($p<0.016$). Three mechanisms are proposed to account for this result: i) Collateral sprouting of inputs to the DVN, ii) Changes in synaptic efficacy of DVN cells receiving inputs directly of indirectly from the spinal cord, and iii) Changes in levels of activity in DVN-input pathways directly or indirectly from the spinal cord.

This research was supported by grants to Professor T.H. Bullock from the NASA, NSF and NIH.

1726 WHAT ACTIVATES VESTIBULO-OCULAR ADAPTATION TO VISION REVERSAL? G. Melvill Jones and G. Mandl. Aviation Med. Res. Unit, McGill University, Montreal, Canada H3G 1Y6.

Previous experiments have shown that optical reversal of vision during head movement in normal light leads to marked adaptive changes in the (opposed) vestibulo-ocular reflex (VOR). There appear to be two possible sources of physiological drive for this adaptation: (1) conflict between the reversed visual and normally acting (ie opposed) vestibular inputs to the oculomotor system and (2) retinal image slip due to incomplete resolution of this conflict.

To investigate the adaptive significance of these factors, 4 human subjects wearing dove-prism reversing goggles were continuously exposed to strobe light at 4 Hz flash frequency whilst moving as best they could about the laboratory for 5 consecutive hours. Flash duration of the strobe was sufficiently short to prevent significant retinal image slip. One subject had participated in the previous experiments conducted in normal light. The degree of adaptation was tested A) by measuring VOR gain (eye angle/head angle; with mental arithmatic) during sinusoidal head rotation (1/6 Hz; 60° amp.) in the dark; and B) by a sensitive subjective test for image slip without vision reversal ("blur" test) whilst shaking the head at 3 Hz in normal light. Both tests were performed before, and at the end of, the 5 hour experiment in strobe light.

The results of test A) showed a significantly lower gain attenuation (20%) than had previously been found after 5 hours of vision reversal in normal light (50%). The "blur" test, always positive after 10-15 min vision reversal in normal light, was uniformly negative after 5 hours visual reversal in strobe light. Furthermore, "motion" sickness although always manifest after 10-15 min vision reversal in normal light, was never present during the strobe experiments. These latter two findings raise the possibility that the 20% VOR attenuation seen in strobe light was artifactual, perhaps due to lack of arousal associated with subject "fatigue". Thus VOR adaptation is much reduced (possibly absent) when image slip is prevented by strobe light. In a companion communication (Mandl, G. & Melvill Jones, G.) it is shown that reversed pursuit eye movements are nevertheless still present in the strobe light condition. It is therefore concluded that image slip, rather than the presence of conflicting oculomotor drives, probably provides the main source of long-term VOR adaptation to vision reversal.

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1727 EFFECTS OF ATTENTION REQUIRING TASKS ON VESTIBULAR NYSTAGMUS Paul Kileny*, Brian F. McCabe* and Jai H. Ryu. Depts. Speech Path/Audiol. & Otolaryngology, U. of Iowa, Iowa City, IA 52242.

The purpose of this investigation was to determine the influence of two types of attention requiring tasks on vestibular nystagmus elicited by caloric stimulation: mental arithmetic and a short conversation consisting of brief questions and answers. The nystagmus parameters measured were slow phase velocity and beat frequency. The subjects were divided into four groups according to stimulus paradigm and task.

Group A: Fifteen subjects undergoing monaural or binaural 5 cc ice water calorics were instructed to perform a mental arithmetic task. When slow phase velocity reached its peak, mental arithmetic was discontinued and a short conversation was initiated. An increase in slow phase velocity occurred with the initiation of the conversation. Beat frequency remained unchanged.

Group B: Six subjects underwent monaural 2 cc ice water calorics repeated up to 50 times at 30 sec intervals. No attention requiring task was assigned during the trials with the exception of a short conversation initiated with the last stimulus. Stimulus repetition resulted in a decline in slow phase velocity, reversed with the initiation of the conversation. There was no change in beat frequency.

Group C: Six subjects were subjected to monaural 2 cc ice water irrigations repeated up to 50 times at 30 sec intervals. The task was mental arithmetics. There was a significant slow phase velocity decline in three subjects. Beat frequency did not change significantly in all subjects.

Group D: The stimulus paradigm utilized for the six subjects. included in this group was identical to the one utilized in groups B and C. The attention requiring task consisted solely of conversation. No significant response decline occurred in this group with stimulus repetition.

There was a tendency in all subjects for a right or left conjugate lateral eye deviation to occur at the beginning of conversation periods. This eye deviation appeared as a baseline shift.

1. Our results reaffirm the strong dependence of vestibular nystagmus on the general arousal reaction.

2. Conversation appears to be more effective and predictable in maintaining or re-eliciting the general arousal reaction necessary for the prevention of vestibular suppression.

3. It is generally recognized that a spontaneous conjugate lateral eye deviation is one of the accompaniments of the general arousal reaction. To the degree this occurred in our subjects, it is further evidence that conversation is an elicitor of the general arousal reaction.

1728 PROGRESSIVE PATHOPHYSIOLOGICAL CHANGES IN THE INNER EAR OF THE SQUIRREL MONKEY DURING THE FIRST YEAR FOLLOWING RAPID DECOMPRESSION. Jack P. Landolt, Ken E. Money*, E.D.L. Topliff*, A. Nicholas* Def & Civil Inst. Env. Med., P.O. Box 2000, Downsview, Ont. Canada, M3M 3B9, and W.H. Johnson* Dept. Otolaryng. Univ. Toronto, Toronto, Canada.

Squirrel monkeys were tested for vestibular function and then decompressed from 274 meters of sea water (msw). A discrete inner ear "hit" was identified by the sudden onset of spontaneous nystagmus and gross motor instability during the decompression phase of the dive between 61 msw and the surface. Following such a successful dive, the monkeys were tested more formally for vestibular function and then sacrificed for histological examination at intervals from one hour to one year after the dive.

Up to about 7 days after a successful dive, the monkeys show some form of motor instability. Furthermore, electronystagmographic tests indicate the presence of strong spontaneous and positional nystagmuses, and reduced sensitivity to post-rotatory stimulation. Histological sections of the temporal bones of these monkeys show a dense precipitated material (probably blood proteins), strongly agglutinated to the cupula. This is likely responsible for the positional effects encountered. Though hemorrhagic incidents appear in the cochlea (mainly as a result of tissue damage in the scalae) in animals sacrificed within 48 hours after a dive, this is not the case in the vestibular apparatus. When hemorrhage does occur in the vestibular apparatus (> 48 hours after a dive), it always appears in the perilymphatic spaces, and usually close to the region where nerve fibers enter the neuroepithelia. Formations of new connective tissue and bone in tissue-damaged regions are quite evident in monkeys sacrificed 2 months or more following decompression. In these long-term survival monkeys, there is no evidence of position nystagmus; and, furthermore, there is an apparent recovery of the post-rotatory nystagmus even though there is still evidence of pathology in the end organs.

1729 CONDUCTION VELOCITY AND OTHER PHYSIOLOGICAL PROPERTIES IN CAT'S HORIZONTAL CANAL PRIMARY AFFERENTS. Charles H, Markham, Toshiaki Yagi* and Norman E. Simpson*. Dept. Neurol., Sch. Med., UCLA, Los Angeles, CA 90024.

The conduction velocity and other physiological characteristics of the first order horizontal canal afferents were studied in cats anesthetized with pentobarbital. From their spontaneous discharge patterns, neurons were classified into three groups: regular, intermediate and irregular. The irregular units tended to have a low resting rate, high sensitivity to constant angular acceleration, frequently exhibited stimulus adaptation during prolonged acceleration, and showed a short latency from the time of electric stimulation of the labyrinth to recording the action potential near Scarpa's ganglion. The regular units tended to have a high resting discharge rate, low sensitivity, were mostly non-adapting, and showed longer latency to electric stimulation. The intermediate units had a mixed character of regular and irregular units.

Wersäll (1956) and Dunn & O'Leary (1976) have shown thickest afferent nerve fibers terminate in the sensory epithelium on the summit of the crista, thin fibers on the slope, and intermediate size fibers in both areas. Since the conduction time is due predominantly to conduction in the first order axon, and since there is a direct linear relation between conduction velocity and fiber diameter in the medullated nerve fibers, we conclude that the regular firing neurons with thin fibers innervate receptors on the slope of the crista, the irregular neurons with thick fibers go to the summit of the crista, and the intermediate units with medium caliber fibers innervate both the slope and simmit of the crista ampullaris.

1730 DISPLACEMENT OF THE SEMICIRCULAR CANAL CUPULA DURING SINUSOIDAL ROTATION. Jay W. McLaren and Dean E. Hillman, Dept. of Physiol. & Biophys., Univ. of Iowa, Iowa City, IA and Dept. of Physiol. & Biophys., NYU Med. Sch., N.Y., N.Y. 10016.

The cupula has typically been described as a gelatinous structure which is displaced by endolymph as if it were hinged at its receptor cell base. During compression of the canal wall, cupulae that have been injected with a dye track move as diaphragms with greatest displacement near the central axis of the ampulla. (McLaren, J.W. and Hillman, D.E.; Neurosci. Abst. 2: 1060). In this study, displacement of the horizontal semicircular canal cupula of the bullfrog was observed over a physiological range of sinusoidal rotation. A glass micropipette was inserted through the cupula from apex to crista and withdrawn with simultaneous pressure injection of oil droplets darkened with Sudan black. The distances between the opaque droplets in the cupula and reference markers fixed to the ampulla were then measured using frame by frame analysis of high speed motion pictures that were made during sinusoidal rotation of the frog. The results show that the cupula moves through a distance of 8-14 µm for angular velocities of about 200°/sec. (± 40°; 0.75Hz). The region of greatest displacement was approximately 200 µm from the crista, just above its thin, non-planar portion. Displacement was less from this point to the crista and to the apex. When graded angular velocities less than 200°/sec. were applied, measured cupular displacement was proportionally lower. It is concluded that during physiological angular motion, the cupula functions as a diaphragm with maximal displacement close to the crista rather than the apex. (Supported by USPHS, grant NS-13742 from NINCDS).

1731 VESTIBULO-OCULAR RESPONSES IN THE RHESUS MONKEY FOLLOWING PROLONGED OPTICAL REVERSAL OF VISION. F.A.Miles, D.J.Braitman* and B.B.Eighmy*. Lab. Neurophysiology, NIMH, Bethesda, MD 20014.

Two rhesus monkeys were fitted with dove prism spectacles to provide left-right reversal of vision (cf., Gonshor & Melvill Jones, J. Physiol. 256, 381, 1976) and placed in a primate chair allowing free head movement. Horizontal vestibulo-ocular reflex (HVOR) gains (=eye velocity/head velocity) were assessed by measuring eye movements during passively imposed sinusoidal oscillations of the whole body about a vertical axis in total darkness. The HVOR gain of the first animal declined to about 1/3 normal over a 9-day period, at which level it remained for the rest of the experiment (8 weeks). Only minor phase changes (lag) were observed. The second animal received additional daily sessions of forced oscillation about the vertical axis; its HVOR gain fell almost to zero over a 2-week period, at which level it remained for several weeks before increasing but now in the "reversed" direction. The vertical VOR remained unchanged. With 0.4 hz sinusoids (±20°), the HVOR gain reached a maximum of 0.47 with eye velocity almost exactly in phase with chair velocity. However, further tests revealed that these "reversed" responses have unusual dynamic characteristics, often giving way to normally directed responses under some conditions, and are of very limited functional significance: 1. With lower frequency sinusoids eye velocity became markedly phase advanced on head velocity, and with alternating velocity steps (triangle waveform oscillations) the "reversed" responses showed roughly exponential decays with time constants of 0.6-1.0 sec. This contrasts with time constants in excess of 20 sec in both normal monkeys and those that have undergone "pure" VOR gain changes as a result of wearing telescopic spectacles. 2. Caloric nystagmus induced by cold water irrigation frequently commenced in the "reversed" direction but rapidly reverted to the normal after only a second or two, eventually achieving peak slow wave velocities about 1/3 normal. These brief "reversed" responses were at best very weak and easily overlooked, but since they were never observed in the normal animal they are unlikely to be due to some spurious thermal transient in the irrigation fluid. 3. Self-generated head turns in total darkness (measured by coupling the head to a rotary potentiometer) were usually accompanied by "reversed" compensatory eye movements. However, even with the largest head movements (up to 100°) these "reversed" eye movements were invariably small (rarely more than 6°) and always transient, terminating within 0.3-0.6 sec even if the head movement was still in progress. Indeed, when a head movement did continue beyond this time, a second period of ocular "compensation" was often evident in which the eyes now moved, albeit weakly, in the normal direction (i.e., opposite to the head), so that the overall ocular response was biphasic.

1732 COMPARISON OF DYNAMIC RESPONSE CHARACTERISTICS OF AMPULLAR ENDOLYMPHATIC POTENTIALS AND FIRST-ORDER AFFERENTS IN THE ISOLATED GUITARFISH SEMICIRCULAR CANAL. Dennis P. O'Leary and F. Owen Black, Dept. of Otolaryngol., Div. of Vestibular Disorders, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA. 15213.

Dynamic response characteristics of ampullar endolymphatic potentials (AEPs) were studied by recording them simultaneously with first-order afferent spike trains during stimulation with rotational acceleration. One or two afferent units were maintained for multi-hour recordings using forceps electrodes. Beveled micropipettes filled with concentrated salt solutions or endolymph from the contralateral utricle were inserted through the ampullar wall along a vector that bypassed the cupula. Steady state AEPs were in the range +(4 to 10) mV, relative to indifferent electrodes in the perilymph. White-noise rotational acceleration stimuli were applied over a .04 to 4 Hz bandwidth, and cross-correlated with both the AEPs and afferent responses to obtain linear system characteristics. Afferent sensitivities and system characteristics remained stable before, during and after insertions of AEP pipettes, implying minimal receptor damage. AEP response dynamics were fitted by overdamped, second-order system equations of significantly lower bandwidths than those described for afferent responses from this receptor (O'Leary, et al, J. Neurophysiol., 39: 645, 1976). These results are important for modeling transduction and afferent encoding mechanisms in the semicircular canal.

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1733 EFFECTS OF LINEAR ACCELERATION ON SINGLE UNITS IN RAT VESTIBULAR NUCLEI. A. A. Perachio, D. S. Miller*, D. Rice* and W. Bouris, Yerkes Primate Center, Emory Univ., Atlanta, GA 30322.

Characteristics of the dynamic responses of neurons in the vestibular nuclei of urethane/ketamine anesthetized rats have been examined during sinusoidal vertical acceleration of the head over a frequency range of 0.2-0.5 Hz. The vestibular stimulus, which was presumed to act mainly on macular receptors of the saccule (Fernandez, C. et al., J. Neurophysiol., 35, 1972; Spoendlin, H. H., NASA SP 77, 1965), consisted of a series of single cycle, up-and-down, translational movement, at a selected frequency over a fixed distance (17-19 inches). The axis of motion was orthogonal to the horizontal plane of the stereotaxic frame. The head of the animal was fixed in a stereotaxic instrument with the nose pitched downward at an angle of 20°. Single units were found to respond over the full range of acceleration (+0.07 to +0.5 g). Neurons were classified as Z negative sensitive if their activity increased when acceleration was directed upward and were labelled Z positive if firing increased during downward acceleration.

To date, 12 Z positive and 9 Z negative cells have been analyzed. There did not appear to be a characteristic resting level of activity distinguishing either type of cell. No recorded units exhibited regular activity. Coefficient of variation (C.V.) indicated that Z axis cells had low resting rates and fired irregularly, e.g., a typical Z negative unit had a mean resting frequency of 5.7 ± 1.5 impulses/sec, C.V. = .532; and a typical Z positive unit had a mean resting frequency of 11.5 + 3.1 impulses/sec, C.V. = ,235.

Although most recordings were made from the region of the superior and lateral vestibular nuclei, Z axis sensitive units have also been found in the medial vestibular nuclei and ventrally in the region of the medullary reticular formation. Of the spontaneously active cells recorded in the vestibular nuclear complex, Z axis cells represented less than 10% of the sample.

1734 RESPONSES OF MEDIAL VESTIBULAR NUCLEUS NEURONS DURING VESTIBULAR NYSTAGMUS. Robert H. Schor, Shozo Nakao*, Hiroshi Shimazu*. Inst. Brain Research, Univ. Tokyo Med. Sch., Tokyo, Japan.

One of the excitatory inputs to lateral rectus motoneurons comes from the contralateral medial vestibular nucleus (MVN). Axon recordings within the abducens nucleus suggested that the spike activities of MVN neurons are modulated during vestibular nystagmus. Extracellular spike discharges of single MVN neurons were recorded in encéphale isolé cats, under local anesthesia (EEG monitoring showed alternating fast waves and spindle bursts). Units were chosen which showed a clear response to rotation in the horizontal canal plane. Many units having a type I response to rotation (increased firing with ipsilateral rotation) exhibit a rhythmic modulation of their firing rate in association with nystagmus elicited by rotation or electrical stimulation of the labyrinth. The most prominent aspect of this rhythm is an abrupt cessation of firing during the quick inhibitory phase of the contralateral lateral rectus motoneurons. Those MVN neurons which could be activated antidromically from the contralateral abducens nucleus almost invariably exhibited such rhythms.

Recordings were also obtained from type II units (increased firing with contralateral rotation) which were activated at short latencies (3-4 msec) from the contralateral labyrinth. Many of these units also have a nystagmic rhythm, showing an increase in activity at the quick inhibitory phase of the contralateral abducens nucleus. Simultaneous recordings from both type I and type II vestibular neurons show that the increased activity of type II neurons corresponds to the pause of type I neurons during the quick inhibitory phase.

Such type II neurons may be inhibitory interneurons of the crossed commissural inhibitory pathway between the vestibular nuclei. In order to observe the possible synaptic effects of such type II neurons, the membrane potential of type I neurons was averaged using extracellular type II spikes as a trigger signal. With this technique, it was possible to reveal an IPSP with monosynaptic latency, suggesting that the type II neuron directly inhibited the type I cell. Thus a periodic inhibition mediated by type II neurons contributes to the rhythmic nystagmic modulation of type I neurons projecting to the contralateral abducens nucleus.

1735 NATURAL VESTIBULAR STIMULATION: EFFECT ON NEURAL ACTIVITY IN THE MESENCEPHALON OF THE FROG. Barry Skarf. Aviation Medical Res. Unit, Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6.

The adult frog has well developed optokinetic and vestibulomotor reflexes which apparently operate to stabilize the visual image on its retina. It seems likely, therefore, that this animal would need to integrate information concerning head and whole body movement with the visual signals that are received in its primary visual center, the mesencephalic optic tectum.

To investigate whether vestibularly-evoked signals project to the mesencephalon of the frog (Rana pipiens), single and multiunit potentials were recorded extracellularly from the tectum and subtectum of curarized animals (appropriate topical anesthesia having been used). Vestibular influence was inferred when neural activity could be systematically modified by sinusoidal rotation of the animal about a vertical axis in the dark. Frequency of oscillation varied from 0.1-0.5 Hz and peak to peak angular displacement between 20 and 90°. A variety of influences were noted. Vestibular stimulation occasionally resulted in suppression of the "spontaneous" neural activity which was often present in the absence of visual or vestibular stimulation. More frequently, however, there was an increase in impulse frequency during periods of stimulation. Most vestibularly-evoked responses displayed a periodic component of neural activity which was either superimposed on the overall excitatory or suppressive effect or which, in itself, was the predominant characteristic of the response. Both unimodal (ie, one peak of activi-ty per cycle) and bimodal periodic responses were observed.

Units responding to this type of natural vestibular stimulation were found infrequently and only in the deep layers of the tectum and subtectum. In each case, simple manually controlled visual stimuli were also effective in evoking responses at the same recording site. In order to demonstrate an interaction between the two sensory modalities, visual cues complementary to the vestibular stimulus were added by rotating the animal while it was surrounded by a stationary illuminated optokinetic pattern. This type of combined optokinetic and vestibular stimulation enhances the behavioural response to rotation in the unparalyzed animal. Generally, the effect of combined stimulation on the neural unit response was to strengthen its periodic characteristics when compared to the response elicited by exclusive vestibular stimulation.

These observations establish that there is a close association of visually- and vestibularly-evoked signals in the frog mesencephalon and suggest that these two sensory inputs may interact in a functionally significant manner there.

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1736 THE NEURAL SIGNAL OF ANGULAR HEAD POSITION IN CAT PRIMARY AFFERENT VESTIBULAR NERVE AXONS PRESUMED TO INNERVATE THE SACCULUS. David L. Tomko and Robert J. Peterka. Dept. Pharmacology, Sch. of Med., Univ. of Pittsburgh, and Biomedical Engineering Prog., Carnegie-Mellon Univ., Pittsburgh, Pa. 15261.

The response of single eighth nerve neurons to head positions through 360° of rotation was determined in barbiturate anesthetized cats. A polarization vector orientation was calculated for each neuron (Fernandez, Goldberg & Abend, 1972; Loe, Tomko, & Werner, 1973). In the present study, all canal afferents encountered in a penetration were typed to insure that the recording procedures sampled from both the superior and inferior divisions of the nerve, hence from the afferents innervating both the utriculus and sacculus.

It was found that in the cat, as has been reported for the squirrel monkey, there are two populations of polarization vectors; The first, which we reported earlier (Loe, Tomko, & Werner, 1973), lie in a plane tilted by approximately 30° with respect to the Horsley-Clarke horizontal plane, and these units most commonly occur in penetrations where horizontal and anterior canal units are encountered. The orientation of this plane corresponds in general to that of the utriculus and the horizontal semicircular canal. The second population lies in a plane which is tilted by about 30° from the midsagittal plane, and these units most commonly occur in penetrations where posterior canal units were encountered. This second population of afferents is presumed to be made up of neurons which innervate receptor cells of the sacculus. (Supported by NIH grant NS12308).

Fernandez, C., Goldberg, J., & Abend, W., J. Neurophysiol., 1972, 35, 978-997.
Loe, P., Tomko, D., & Werner, G., J. Physiol. (Lond.), 1973, 230, 29-50.

1737 RESPONSES OF VESTIBULAR 2ND ORDER NEURONS IN THE CHINCHILLA DURING SINUSOIDAL OSCILLATION IN THE PRESENCE AND ABSENCE OF A VISUAL INPUT. J. A. Winfield*, P. D. Daniels*, J. Kimm (SPON: J. Miller). Univ. of Washington, Seattle, WA 98112.

Compensatory eye movements in the chinchilla exhibit phase leads and lower amplitudes of modulation relative to normalized head position in the dark at frequencies of rotation below 0.2 Hz (Daniels et al., this issue). Based on these observations an investigation of the dynamic properties of vestibular nuclei neurons was undertaken with similar stimulus conditions. Chinchillas were implanted with horizontal EOG electrodes and a recording chamber was fixed to the skull over the brainstem vestibular area. The response characteristics of simple cells were recorded at frequencies from 0.06 Hz to 1.0 Hz in the presence and absence of visual input.

Two basic categories of unit responses were observed, 53% were classified as Type I and 47% as Type II cells. These units could be further subdivided as follows:

1) VESTIBULAR + EYE MOVEMENT RELATED CELLS: 66% of the unit population responded with peak firing rates in phase with maximum head velocity. In addition, these cells burst prior to saccades in the direction opposite to the direction of vestibular activation; i.e. a Type I cell in the right brainstem bursts to a left saccade.

2) VESTIBULAR ONLY CELLS: The remaining 33% of the cells had their peak firing occurring at about maximum head velocity, but showed no relationship to any type of eye movements.

Only those units where activity were correlated with eye movement showed light-dark differences in their response patterns. The types of changes most notable were:

1) DC THRESHOLD SHIFTS: During periods of oscillation in the dark, there was a decrease in the amplitude of modulation, due to a concomitant equal decrease in spontaneous firing rate. That is, the same sinusoid could fit both the light and dark responses with a DC shift accounting for the lower firing rates observed in the dark.

2) GAIN CHANGES: Additionally, other units increased their firing rates during oscillation in the light.

Some of these units exhibited both effects, however, no units showed any significant change in phase for either lights on or off.

A model was formulated to account for the observed phase and gain shifts occurring during low frequency oscillation in the dark based on these non-linear unit responses.

1738 THE VESTIBULO-OCULAR RESPONSE TO SMALL AMPLITUDE AND HIGH FREQUENCY ROTATIONS IN THE RABBIT. Barbara J. Winterson*, Han Collewijn*, and Robert M. Steinman. (SPON: Frank Baker) Dept. Physiol., Erasmus Univ. Rotterdam, the Netherlands and Dept. Psychol., Univ. of Md., College Park, Md. 20742

Dutch-belted rabbits were rotated sinusoidally on a torsion swing with eyes covered (darkness) and with eyes open (light) while their eye movements were monitored (noise level = 18" arc) by an implanted search coil in a magnetic field. Torsion swing amplitudes ranged from 30' arc down to zero, and frequency ranged from 0.22 Hz to 11.5 Hz.

The vestibulo-ocular response in the dark (VOR) was always observed, that is, the VOR was evident until it could not be distinguished from small (< 5') spontaneous drifts of the eye. Compensation provided by VOR was not perfect. Mean gain (amplitude of VOR/amplitude of swing rotation) ranged from 0.19 at the lowest frequency and was never better than 0.45 at higher frequencies. The VOR to small amplitudes, although far from perfect showed signs of linearity in that gain was not influenced systematically by amplitude.

In the light slow compensatory eye movements were more effective for frequencies up to 3.7 Hz. Gain ranged from 0.42 to 0.52 and phase leads were diminished under this condition. Compensation was not improved at the highest frequencies (9.0 Hz and 11.5 Hz).

We conclude that the vestibulo-ocular response in the rabbit to small rotations is not fundamentally different from the vestibulo-ocular response to large rotations (Baarsma & Collewijn, 1975). However, the VOR to small, as well as large, amplitude rotations is insufficient to stabilize the retinal image, even when it is allowed to work with visual input.

Similar results have been found in the human where compensation provided by VOR, even with visual input, is not sufficient to stabilize the retinal image perfectly (Winterson, Steinman, Skavenski, Hansen & Robinson, 1975). These results raise visual and perceptual questions because visual acuity and perceptual stability cannot arise directly from the perfection of oculomotor compensation.

1739 A CONFLICT MODEL FOR VISUAL AND VESTIBULAR INFLUENCES ON PERCEPTION OF ROTATION ABOUT THE VERTICAL. Greg L. Zacharias* and Laurence R. Young, Dept. Aero & Astro, M.I.T., Cambridge, MA 02139.

Subjects in a moving-base flight trainer were given control over their own rotation about the vertical, and were asked to keep themselves stationary in space. A pseudo-random wide-band vestibular stimulus was applied to the trainer drive. A visual motion cue was simultaneously given the subject via a moving stripe pattern projected on the trainer's side windows, in the subject's visual periphery. Three types of visual cues were used: constant speed, space stationary, and pseudo-random. Subject response was analyzed in the time and frequency domains to infer motion sensation dependence on combined cue presentation. The results confirm low-frequency visual cue dominance (circularvection) and high-frequency vestibular dominance, and support a non-linear visual-vestibular "conflict model" (see below). This parsimonious descriptive model is shown to be consistent with unit recordings in the monkey vestibular nucleus, under similar stimulus conditions.

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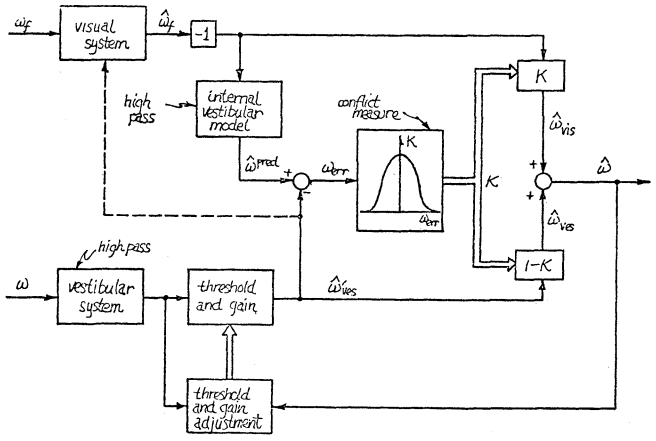

PARALLEL CHANNEL CONFLICT MODEL

1740 COMPARISON OF ANTERIOR AND POSTERIOR VIIITH NERVE INPUT TO THE GOLDFISH MAUTHNER CELL. Steven J. Zottoli. Res. Inst. on Alcoholism, Buffalo, N.Y. 14203 and Dept. of Physiology, SUNYAB.

A comparison was made of excitatory post synaptic potentials (EPSPs) evoked by stimulation of the anterior and posterior branches of the VIIIth nerve in the goldfish Mauthner cell (M-cell). Intracellular recordings were made with KCl and K_2SO_4 electrodes positioned in the M-cell soma and the lateral dendrite up to 400 μm lateral to the axon cap. As has been previously found, stimulation of the posterior VIIIth branch evoked two main peaks. The first is a short latency (< 0.2 msec) electrotonic EPSP which increased in amplitude (up to 40 mV) laterally; this finding is consistent with the postulate that this potential originates from myelinated club endings located on the distal lateral dendrite (Furshpan, Science. 144:878, 1964). This electrotonic PSP, which is sufficiently powerful to excite the M-cell, is followed by a longer latency monosynaptic chemically mediated EPSP. In contrast, predominantly chemical PSPs were evoked on stimulation of the anterior VIIIth branch. In some cases this response was preceded by an electrotonic component no more than 10-15% the amplitude of the chemical component. The chemically mediated component was capable of bringing the M-cell membrane potential to the firing level, at high stimulus strengths.

Goldfish display a rapid M-cell initiated startle response to sound. Their inner ears consist of three semicircular canals and at least three otolithic organs, the sacculus, lagena and utriculus. The posterior branch of the VIIIth nerve arises in part from the first two, which are primarily auditory receptor organs, while the anterior branch contains afferents from the utriculus, which is more involved in the maintenance of equilibrium. This behavioral and morphological evidence is consistent with the observed differences between anterior and posterior VIIIth nerve inputs to the M-cell. Although input activity in both might be expected to modulate M-cell excitability, synchronous activity in the latter is more likely to produce the rapid excitation of the M-cell underlying the startle response. (Supported in part by NIH Postdoctoral Fellowship No. F32 NS 5282 and Grant No. NS-12132).

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1741 A SUBDIVISION OF EFFERENTS FROM STRATUM GRISEUM SUPERIFICIALE IN THE TREE SHREW SUPERIOR COLLICULUS. J.E. Albano, T.T. Norton and W.C. Hall, Dept. Anatomy, Psychology and Physiology, Duke Univ., Durham, North Carolina 27710

The superficial layer of the superior colliculus, stratum griseum superficiale, can be subdivided into at least two sublamina in the tree shrew, Tupaia glis. An upper sublamina contains a population of small neurons which have small receptive fields and respond briskly both to the onset and offset of stationary and to moving stimuli. A lower sublamina contains a mixture of large and small cells which exhibit several types of receptive field organization. In general, these cells have larger receptive fields than those in the more superficial sublamina and respond only to moving stimuli (Albano, Humphrey & Norton, Anat. Rec. 181: 299, 1975; Albano, Ph.D. Thesis: Duke Univ, Univ. Microfilm,1977).

The present experiments were designed to determine whether these sublaminae can also be distinguished on the basis of their efferent connections. To answer this question, 0.1 to 0.2 µl of 40% horseradish peroxidase dissolved in saline was injected into three known diencephalic targets of the superficial layers of the superior colliculus in the tree shrew, the dorsal and the ventral lateral geniculate nuclei and the pulvinar (Harting, Hall, Diamond & Martin, JCN, 148: 368, 1973).

Following injections in either the dorsal or the ventral lateral geniculate nucleus, horseradish peroxidase reaction product was found predominantly in the small cells in the upper sublamina. In contrast, following injections in the pulvinar, the reaction product was restricted primarily to the large cells in the lower sublaminae.

Taken together, these results suggest that two visual relays to cortex, the pulvinar and the dorsal lateral geniculate, can be distinguished on the basis of the kinds of information they receive from the superior colliculus. The dorsal and ventral lateral geniculate nuclei, on the other hand, may be receiving similar kinds of information from a relatively homogeneous population of colliculus cells.

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1742 THE RETINOTOPIC ORGANIZATION OF THE SECOND VISUAL AREA IN THE CAT. K. Albus[x] and R. Beckmann[x] (SPON: B.B.Lee). Max Planck Instit.f.Biophys.Chemistry, Dept.Neurobiol. 34 Göttingen, Fed.Rep.Germany

The representation of the horizontal meridian (HM)and the upper visual field (VF) in the second visual area (V2) of the cat have been controversial topics. We have been studying the extent and the location of V2 in anesthetized (Nembutal) and paralyzed (Flaxedil) cats using single and multiple unit recordings.Results:On the lateral gyrus (LG) the border between V2 and the third visual area (V3) represents the periphery of the lower VF. At anterior Horsley Clarke levels the border runs along the top of the LG,turning down - at posterior Horsley Clarke levels - the lateral flank of the LG.The representation of the area centralis in V2 extends from the transition zone between LG and postlateral gyrus (PLG) some mms posteriorly on the lateral flank of the PLG. From this cortical area the representation of the HM spreads out in a latero-posterior direction covering the bottom of the postlateral sulcus (PLS) and the medial bank of the posterior suprasylvian gyrus (PSSG). Characteristically the receptive field (RF) centres at the HM cover up to 50 degrees eccentricity in the VF, whereas in the adjacent lower and upper VF representation they cover only up to 20 degrees. At the lateral end of the HM representation a reversal in RF positions indicating the existence of V3 was seen in only one out of 3 cats. The upper VF is found to be represented in the latero-ventral extension of the posterior PLS(PPLS). In the posterior bank of the PPLS that sector of the VF is represented which adjoins the vertical meridian. In the anterior bank of the PPLS that sector of the VF adjoining the HM is represented. The remaining part of the VF occupies the bottom of the PPLS. Exploring the upper part of the anterior bank of the PPLS and the posterior part of the PSSG a reversal in RF positions indicating V3 was not found. However proceeding further down the PPLS and the posterior PLG in a latero-ventral direction the RF positions were found to move back from the upper VF periphery to the area centralis. It is likely that this cortical region laterally adjoining V2 is V3 and that therefore the border between V2 and V3 on the posterior PLG represents the periphery of the upper VF. We conclude that in the cat's visual cortex there is a second mirrored relationship across the periphery of the VF, forming the boundary between V2 and V3.

1743 BINOCULAR RIVALRY: AN EXPERIMENTAL PARADIGM FOR THE STUDY OF THE PHYSIOLOGY OF PERCEPTION. J. M. Allman, J. Myerson and F. M. Miezin*. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

In sensory neurophysiological experiments, the organism typically is treated as a passive object. We envision an alternative approach in which one asks the question: where in the nervous system do the responses of neurons covary with changes in the perception of the stimulus and not just with changes in the physical stimulus. In binocular rivalry, when one eye views bars moving in one direction and the other eye views bars moving in the opposite direction, the perceived scene alternates abruptly every few seconds from what one eye sees to what the other eye sees, and the bars appear periodically to reverse their direction of movement. We have trained a monkey, Macaca fascicularis, to report changes in the direction of movement of bar gratings by tapping one of two keys. We did this in order to test the feasibility of recording from single neurons in visual cortex while monkeys experience binocular rivalry and report their ongoing changes in perception. During the initial training, each eye viewed a separate oscilloscope with gratings moving in the same direction for both eyes. Following training, rivalry-inducing stimuli (gratings moving in opposite directions for each eye) were presented on approximately 40% of the trials. Rivalry presentations were terminated with catch trials by reversing the direction of one of the gratings so that both eyes viewed movement in the same direction. Stimuli ranged from 2 to 8 degrees/sec in velocity and from 0.5 to 1.5 cycles/degree in spatial frequency. Rate of perceived alternation in direction of movement increased as a function of velocity and spatial frequency for the monkey and for human subjects tested with the same procedure. Gamma functions with nearly equivalent parameter values describe the distributions of rivalry phase durations in both monkey and man. These functional similarities between binocular rivalry in monkey and man are evidence of the accuracy of the monkey's reports of his perceptions. The rivalry-inducing stimuli which we have used permit the testing of hypotheses concerning underlying neural mechanisms. The waxing and waning of activity in cortical neurons showing directional selectivity or differential responsiveness to monocular stimulation, if correlated with reported perceptual changes, would be evidence for selective facilitative and/or suppressive processes.

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1744 BINOCULAR INTERACTIONS IN STEADY STATE VISUAL EVOKED RESPONSES. P. Apkarian,* K. Nakayama,* C. W. Tyler* (SPON: C.K. Peck). Smith-Kettlewell Institute of Visual Sciences, University of the Pacific, San Francisco, CA 94115.

The steady state visual evoked response (VER) was measured with sinusoidal gratings which were temporally modulated in counterphase. VER amplitude measures were obtained with a synchronous narrow-band filter of 2% bandwidth (-6db) at a center frequency equal to the reversal frequency between 10 and 50 rps.

Binocular interactions were investigated as a function of spatial frequency, contrast, and retinal location. The response amplitude showed narrow peaks as a function of both spatial and temporal frequency. All peaks recorded showed some degree of binocular summation in comparison to the mean monocular response. Some peaks showed marked binocular facilitation of as much as 4x the mean monocular response. Binocular facilitation tended to be stronger at higher contrasts. Binocular summation occurred for horizontal gratings for which stereopsis is not possible, but facilitation greater than 2x the monocular response was not obtained.

The spatial frequency of the response peaks depended markedly on the retinal location of the stimulus. Thus the degree of binocular interaction varied with different retinal locations. In some cases a single peak could be isolated as deriving exclusively from a retinal location as small as one square degree. The largest VER was usually obtained outside the fovea at all spatial frequencies.

These results indicate a high degree of specificity in the evoked potential as a function of many basic stimulus parameters.

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1745 ANATOMICAL OBSERVATIONS ON THE VENTRAL LATERAL GENICULATE NUCLEUS (LGNv) OF MONKEY. Richard S. Babb, Pedro Pasik and Tauba Pasik. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The LGNv of the monkey (M. mulatta) consists of two distinct laminae. The ventral lamina appears orally within the incoming fibers of the optic tract concurrently with the anterior pole of the LGNd. It is rich in neurons and very poor in myelinated fibers. The dorsal lamina, which is initially separated from the ventral lamina by optic tract fibers, becomes contiguous at more caudal levels, but remains distinct due to its richness in myelinated fibers. Specimens prepared by the Golgi-Kopsch method show at least 3 neuronal types: an elongated cell, 15x10 µm, with dendrites oriented in a bipolar fashion within the coronal plane of the section and bearing twig-like protrusions with enlarged endings; a round neuron, 15 µm in diameter, with long delicate dendrites having smooth outlines and terminal arborizations with bulbous appendages; a larger cell, over 20 µm in diameter, with thick long dendrites exhibiting spine-like protrusions and oriented ventralward, sometimes reaching the LGNd capsule. The distribution of these types is unclear although the smooth dendrite category was observed only in the ventral lamina. The dendrites of the other types may extend into both laminae.

Thin sections from individual laminae were prepared for electron microscopy. They were obtained from oriented blocks where clear identification of the laminae could be made in 1 µm sections of the entire face. Three neuronal types are identified: a fusiform cell, 20x10 µm, with scant cytoplasm, poor in organelles, and with very few axosomatic synapses; a roundish cell, about 18 µm, with a richer perikaryon, light large mitochondria, small stacks of rough endoplasmic reticulum, and up to 6 axosomatic synapses in a given section; a larger neuron, over 20 µm in diameter, with very rich cytoplasm, numerous organelles, small dark mitochondria, many large stacks of rough endoplasmic reticulum, and up to 14 axosomatic synapses in a section. Dendritic profiles exhibit protrusions and spines particularly abundant in the dorsal lamina. Profiles containing synaptic vesicles are at least of 4 types. The most frequently seen has dark mitochondria and mostly round vesicles. A smaller, cup-shaped terminal has dark mitochondria and small spheroidal vesicles. A large ending contains large pale mitochondria and larger spheroidal vesicles. A profile with pleomorphic vesicles shows medium dense mitochondria and occasional cisterns. The latter element exhibits both presynaptic and postsynaptic sites and participates in complex arrangements involving triadic and serial synapses with the other 3 axon terminals and purely dendritic profiles. These complex synapses are more common in the ventral lamina which resembles the dorsal lateral geniculate nucleus. Aided by N.I.M.H. Grant # HM-02261.

1746 THE CHOLINERGIC SYSTEM IN THE DEVELOPING CHICK RETINA. Charles R. Bader*, Janet L. Moore* and Robert W. Baughman. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

In the chicken retina there is a Na dependent, hemicholinium-3 (HC-3) sensitive high affinity (HA) uptake of choline localized to a small number of retinal cells and required for acetylcholine (ACh) synthesis (Baughman and Bader, 1977). In the present work the activity of choline acetyltransferase (CAT) and Na dependent, HC-3 sensitive ACh synthesis were studied during development of the chicken retina. CAT activity per mg protein in retinal homogenates increased 30-fold between embryonic days 7 and 11 and then remained constant through hatching at day 21. The synthesis and storage of ^{3}H ACh, determined following an in vitro incubation in the presence of ^{3}H Ch, however, increased in two stages. The first stage coincided with the increase in CAT activity (days 7-11), and through day 13 ACh synthesis was not blocked when HC-3 was present or when sucrose replaced NaCl in the incubation medium. The second stage occurred between days 14 and 19, but now, as in the adult, ACh synthesis appeared to be linked to HA choline uptake since it was blocked by HC-3 or the absence of Na. The second increase in synthesis is concurrent with synaptogenesis (Hughes and LaVelle, 1974) and the appearance of visual function (Witkovsky, 1963). Thus, in addition to the increase in CAT activity, which is completed relatively early, there is a second process appearing with final maturation, possibly related to HA choline uptake, that is important for synthesis and storage of ACh in the developing chick retina.

1747 QUANTITATIVE RESPONSE PROPERTIES OF NEURONS IN THE DORSOMEDIAL AREA (DM), A THIRD TIER VISUAL AREA IN THE OWL MONKEY. J. F. Baker*, F. M. Miezin*, J. Myerson, W. T. Newsome*, S. E. Petersen*, and J. M. Allman (SPON: V. Lewis). Div. of Biology, California Institute of Technology, Pasadena CA 91125.

The visual cortex immediately anterior to the second visual area in the owl monkey, Aotus trivirgatus, contains five separate representations of the contralateral visual hemifield. We have studied single neuron response properties in one of these third tier areas, the dorsomedial area (DM), in chronically prepared sedated animals. In experimental sessions an animal initially received a tranquilizer, trifluprom azine, followed by hourly doses of ketamine. The eyes were anesthetized locally with dibucaine before fixing them to immobile rings. Computer controlled visual stimuli were presented while recording from single DM neurons with glass insulated platinum-iridium electrodes. Stimuli were moving bars of various orientations, velocities, lengths, and widths presented in pseudorandom order. Responses were calculated as the difference between the mean impulse rate during the stimulus presentation and the mean spontaneous impulse rate. Most DM cells were orientation selective; they responded best to a stimulus of a particular orientation moving in either direction orthogonal to that orientation and gave a less than half maximal response to the orthogonal orientation. A few DM cells were directionally selective; they had a preferred direction of movement and gave a less than half maximal response to the opposing direction. The remaining cells were pandirectional, with all orientations and directions evoking a response at least half the maximum. These categories were not sharply distinct. Instead, our measures showed a continuous distribution from the extremes of orientation selectivity and direction selectivity to pandirectionality. DM cells responded maximally to stimulus velocities of 25 deg./sec or greater and responses were nearly proportional to the logarithm of velocity up to the optimal speed. Responses showed approximately linear summation with increasing stimulus length, usually up to the full extent of the cell's mapped receptive field. Further increases in stimulus length had no effect. Increasing stimulus width beyond an optimal small value (about 1 deg.) either had no effect or resulted in decreased firing. Virtually all DM cells were binocularly activated with the majority of cells driven about equally by the two eyes. Some cells' responses were strikingly affected by variations in horizontal retinal image disparity, and several patterns of disparity tuning were observed. This research was supported by NIH grants NS-12131 and NS-00178 and by Sloan, Spencer, and Weizmann fellowships.

1748 EFFERENT INNERVATION AND CIRCADIAN RHYTHMS IN THE LIMULUS VISUAL SYSTEM. Robert B. Barlow, Jr., and Steven C. Chamberlain.* Institute Sensory Research, Syracuse U., Syracuse, NY 13210

An endogenous clock mechanism significantly influences the response characteristics of the Limulus visual system. The properties of the circadian clock have been studied with physiological, anatomical, and behavioral techniques. The physiological studies show that when Limulus is kept in continuous darkness the electroretinographic and optic nerve responses from the lateral eye are larger at night than during the day. The spontaneous discharge recorded in the dark from single optic nerve fibers also exhibits a circadian rhythm: the rate of the spontaneous discharge is low at night and high during the day. The circadian rhythms in the neural activity of the lateral eyes are mediated by efferent fibers that fire synchronous bursts of impulses at night and few or no impulses during the day. Illumination of the median eyes can modulate the discharge of the efferent fibers and thereby influence the neural activity of the lateral eyes. A comparison of the daytime and nighttime intensity-response functions for a single ommatidium indicates that the efferent input increases both the quantum catch and the gain of the photoreceptors.

Anatomical studies are beginning to reveal the underlying neural organization which mediates efferent control of visual sensitivity and interactions between visual organs. Results of cobalt sulfide-silver studies have shown the detailed morphology of the primary visual inputs to the brain. The extensive innervation patterns of single optic nerve fibers from the median and lateral eyes have areas of overlap. The second optic ganglion receives primary inputs from the ventral, median, and lateral eyes as well as from mechanoreceptors on the carapace. Relationships between these inputs and nearby neurosecretory centers are being studied with HRP and Alcian blue-yellow procedures.

Behavioral studies reveal circadian rhythms in the locomotor activity of Limulus. The animals' nocturnal periods of maximal locomotor activity correspond reasonably well to the period of elevated retinal sensitivity. The circadian changes in the response characteristics of the Limulus visual system may adapt the animal for functioning in dim light, a result supported by preliminary behavioral studies in the field (ocean).

1749 THE FOVEAL LOCAL ERG WITH TRANSIENT AND STEADY STATE FLICKERING STIMULI. W.S. Baron and R.M. Boynton. SRI, Menlo Park, CA 94025 and UCSD, Dept. Psych., La Jolla, CA 92093.

The foveal local ERG (LERG) of the monkey has been shown, over a limited range, to behave in a linear fashion under steady state flicker conditions. Using a criterion response amplitude within this range, its temporal modulation transfer function has been obtained. From these data we have calculated theoretical impulse responses and integrated them over a 400 msec period. Comparisons between these derived responses and experimentally obtained responses to 10% incremental and decremental impulse and rectangular pulse stimuli, shows that the linear Fourier transform does not hold. A b-wave appears to be present in the experimental incremental responses which is not as apparent in the theoretically derived responses, and is absent in the decremental impulse response.

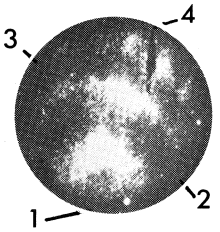
1750 UPTAKE SYSTEMS FOR γ-AMINOBUTYRIC ACID IN THE CHICKEN RETINA. Robert W. Baughman, Thomas L. Schwarz* and Charles R. Bader.* Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

The transport of γ-aminobutyric acid (GABA) and its analogs into cells in the chicken retina was examined. When pieces of retina were incubated in vitro in medium containing ^{3}H GABA at concentrations ranging from 0.05-100 μM and subsequently analyzed autoradiographically, differences in the distribution of GABA uptake were observed. At the lowest concentrations uptake into horizontal and ganglion cells was significant, but amacrine cells were only weakly labelled. In the range 1-25 μM all three cell types were labelled, consistent with previous studies carried out in this range (Marshall and Voaden, 1974). At 100 μM uptake into ganglion cells was negligible compared to the levels observed in horizontal and amacrine cells. In the mammalian CNS 2,4-diaminobutyric acid (DABA) and β-alanine (β-Ala) have been reported to block uptake of GABA into neurons and glia respectively (Kelly and Dick, 1975). In the present study these GABA analogs were used to further characterize the uptake mechanisms in the various cell types. After incubation with ^{3}H DABA (8.3 μM) label was observed in horizontal and amacrine cells, but not in ganglion cells. On the other hand, ^{3}H β-Ala (2.8 μM) was taken up only by ganglion cells and not by horizontal or amacrine cells. As with GABA, neither DABA or β-Ala was taken up by photoreceptors, bipolar cells or Mueller glial cells. A 400-fold excess of DABA effectively blocked ^{3}H GABA uptake into amacrine cells, but even a 5000-fold excess did not effect uptake into horizontal or ganglion cells; a 400-fold excess of β-Ala blocked ^{3}H GABA uptake into ganglion cells, but a 5000-fold excess did not effect uptake into horizontal or amacrine cells. The selective inhibition of GABA uptake observed is consistent with the pattern of DABA and β-Ala uptake, except for the failure of DABA to block GABA accumulation by horizontal cells. Such a complete lack of inhibition was unexpected in view of the high rate with which horizontal cells accumulated ^{3}H DABA. The different behavior for uptake under the conditions described suggests that in the chicken retina horizontal, amacrine and ganglion cells may each possess biochemically distinct transport systems for GABA.

This research was supported by NIH Grant EY00606.

1751 FURTHER OBSERVATIONS ON THE PROJECTIONS OF THE LAYERS OF THE SUPERIOR COLLICULUS IN THE RHESUS MONKEY WITH AUTORADIOGRAPHIC TRACING METHODS. Louis A. Benevento and Michael Rezak. College of Medicine, University of Illinois Medical Center, Chicago, IL.

Two previous degeneration studies(Benevento & Fallon, JCN 160: 339,1975;Partlow et al.,JCN171:285,1977)have produced conflicting results regarding the projections of the superior colliculus(SC) to the dorsal lateral geniculate nucleus(DLG), intralaminar nuclei(IL), posterior nuclear group(PNG), lateral and ventral posterior nuclei, pretectum(PT) and accessory optic nuclei. These conflicts could have been due to the number of SC layers lesioned, the survival time used, or to destruction of axons of passage from the PT. We have attempted to clarify these discrepancies and also add to the previous findings by further examining our autoradiographic material on the projections of the layers of the SC and PT(e.g.,Benevento & Rezak,Brain Res.108:1,1976;Benevento et al. Brain Res.in press:1977). Our autoradiographic studies confirm that the superficial cell layer of the SC projects to the DLG. In addition, heavily labelled fibers were found between all layers of the DLG, and a moderate density of grains was seen among the cells of the magnocellular and parvocellular layers(Fig). The amount of label found in the DLG was too dense to be considered solely due to labelled axons destined for the ventral lateral geniculate(VLG) which contained only a moderate amount of grains medially. Labelled axons destined for the VLG take a direct route through the brachium while those destined for the DLG pass through the inferior pulvinar(PI) where a complex distribution of grains is also found as described before(Brain Res., 1976). The results also show that the retinorecipient superficial cell layer does not project to the IL, but that the deep layers do project to the IL, including part of the centromedian. Thus, it is the deep SC layers, like the PT, which project to visuomotor and arousal systems. Labelled axons were again found in the lateral and ventral posterior nuclei but these now appear to be axons of passage destined for other targets(JCN,1975;Brain Res., 1977). Injections in the superficial cell layer also produced a moderate amount of grains in the deep SC layers, the sublentiform and olivary nuclei, within and about the inferior colliculus and in the dorsal and lateral terminal nuclei(NAOT and "Z" in JCN,1975). In many cases dense label was found in the suprageniculate and limitans nuclei, confirming a SC projection to the PNG.(Supported by NSF Grant BNS 75-07349).



Darkfield of grains between & in DLG layers

1752 PATTERN EVOKED POTENTIALS IN A BLIND HUMAN LACKING VISUAL ASSOCIATION CORTICES. I. Bodis-Wollner, A. Atkin, E. Raab* and M. Wolkstein*, Mount Sinai School of Medicine of CUNY, New York 10029

Visual evoked potentials to diffuse light-flashes, to coarse checkerboards, and to gratings of low spatial frequency were recordable and of normal size in a blind boy. His vision had been normal until age 2 years, when as a consequence of a febrile illness he was left deaf, blind, and slow in developing. While hearing and motor coordination had ultimately returned to normal, at 6 years of age there was still no sign of vision. Ophthalmological examination revealed normal retinae and intact oculomotor and pupillary responses. Computerized axial tomography revealed bilateral destruction of cortical areas 18 and 19, while a strip of occipital cortex on the left corresponding to area 17 was preserved.

A rare opportunity was provided by the following features of this case. At least 2 years of normal visual pathway development occurred prior to blindness. The CT scan and pattern EP are new techniques which were available for correlating anatomy and electrophysiological functions in this living human. In addition we were able to correlate these studies with behavioral observations. The absence of vision, the destruction of areas 18-19, with the apparent preservation of area 17 and the temporal cortex, and the presence of normal flash and low spatial frequency pattern EPs suggests the following consideration. Blindness may result from the destruction of the visual association cortices either because they are per se essential for vision or because they are required as a relay to the inferotemporal cortex. Either hypothesis is noteworthy in view of subhuman primate studies. In subhuman primates, when cortical areas 18 and 19 are destroyed but area 17 is spared, visual acuity and the ability to sort objects are preserved (Denny Brown, D. and Chambers, A.: Arch. Neurol. 33:219, 1976). However, the animals may lose the capacity to learn new discriminations between visual patterns, apparently because destruction of areas 18-19 interrupts the pathway from the primary visual projection cortex to the inferotemporal cortex (Mishkin, M.: in Brain and Behavior, ed. A.H. Karczmar and J.C. Eccles, Springer-Verlag, Berlin-Heidelberg, 1972; pp. 187-208).

In any case it is apparent from this human study that EPs can be elicited by patterns of low spatial frequency when area 17 remains in spite of destruction of the visual association cortex. Furthermore behavioral and electrophysiological indices of pattern detection may no longer be correlated after the visual association cortex has been destroyed.

1753 VISUAL CORTICAL EYE MOVEMENT POTENTIALS AND THEIR ASSOCIATION WITH ALERTING RESPONSES. R.M. Bowker*, J.C. Hendricks*, and A.R. Morrison, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.

We recently demonstrated that during wakefulness the alerting response, which ranges from "sizing up" of stimulus significance to startle, is the main determinant of amplitude of the visual cortical eye movement potential (EMP), not the level of illumination. We now show that EMP are present during alerting, but not during non-alerting behaviors, even in well-lighted conditions. Three cats were chronically implanted for recording EEG,EOG, and neck EMG activity. Transcortical electrodes were placed in the visual cortex. Recordings were correlated with the behaviors observed during food presentation to a fasted cat. Food presentation produced an orientation consisting of a head and neck movement toward the food source and 2-5 scanning eye movements. Each eye movement was accompanied by large EMP. The cat then rapidly approached and consumed the food. Eye movements were present during this goal-directed phase, but EMP were consistently absent. EMP reappeared only during spontaneous searching following food consumption. Daily feeding by repeated presentations resulted in gradual attenuation of EMP amplitudes during orientation. By the fourth day, even though the cat remained highly motivated as evidenced by its persistent ravenous appetite, EMP were no longer discernible above the baseline recording unless a non-specific, uncontrollable stimulus appeared. However, large EMP were always present between trials. With the introduction of novel objects amplitudes returned to equal those recorded during initial training and PGO waves during PS. Other non-alerting behaviors associated with eye movements but not EMP included responses to petting, grooming and food and water consumption. However, when transient scanning interrupted these activities, EMP reappeared. These results demonstrate that under well-lighted conditions EMP appear during alerting behaviors in association with eye movements, but that during non-alerting, goal-directed, and simple learned tasks, visual cortical EMP no longer accompany eye movements. Furthermore, the absence of EMP with these behaviors indicates that contrary to previous suggestions, EMP do not represent simple corollary discharges. Our findings support the idea that they represent an enhancement of the transfer of information in the visual system which would be particularly important during alerting behaviors. These findings are in keeping with our initial hypothesis that the spontaneous PGO wave during PS represents a sign of the activation of an alerting network.

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1754 A VISUAL QUICKIE: A BISYNAPTIC RETINAL PATHWAY TO THE VESTIBULO-CEREBELLUM AND OCULOMOTOR NUCLEAR COMPLEX. N. Brecha, H.J. Karten, Stephen Hunt*, Department of Psychiatry, S.U.N.Y., Stony Brook, N.Y. 11794.

The accessory optic nuclei, which include the nucleus of the basal optic root (nBOR), are readily identifiable in sighted vertebrates. The nBOR in pigeons receives a specific projection from the displaced retinal ganglion cells (Karten, Fite, and Brecha, PNAS, 1977) and projects upon the vestibulo-cerebellum (Brauth and Karten, Exp. Br. Res., 1977), and possibly the oculomotor nuclear complex (Herrick, 1948). These findings suggest that this system plays a role in oculomotor reflexes.

The displaced ganglion cells are located at the margin of the inner nuclear layer and inner plexiform layer of the retina Following injection of horseradish peroxidase (HRP) into nBOR retrogradely labelled displaced ganglion cells were identified and serial sections of these cells examined in the electron microscope. These studies have revealed axodendritic and axosomatic terminals upon the displaced ganglion cells. Some of the somatic endings arise from myelinated axons. No synaptic ribbons were found in the presynaptic profiles. These findings indicate the afferents to displaced ganglion cells probably arise from amacrine cells and centrifugal axons.

Unilateral injections of 20μCi ^{3}H-leucine within nBOR have demonstrated projections upon both the oculomotor nuclear complex and vestibulo-cerebellum. These projections have been confirmed by the injection of HRP into the terminal projection fields of nBOR and subsequent identification of HRP labelled cells within nBOR. The nBOR projects upon the contralateral dorsolateral and ipsilateral ventromedial and ventrolateral nuclei of the oculomotor nuclear complex. Axons of nBOR also join the ipsilateral brachium conjunctivum cerebellopetale, partially decussate in the dorsal cerebellar commissure and terminate bilaterally as mossy fibers within the granule cell layer of folia IXc, IXd and paraflocculus (uvula). Intraocular injection of 850μCi ^{3}H-proline and a survival period of 12 days results in the appearance of longitudinally aligned bands of label within folia IXc, d of the cerebellum. This presumably reflects the terminal distribution of nBOR efferents following transneuronal movement of label from retinal terminals to cell bodies within nBOR.

These findings indicate that displaced ganglion cells constitute a unique population of retinal neurons that give rise to a bisynaptic pathway to the vestibulo-cerebellum and oculomotor nuclear complex.

1755 INTRACELLULAR RECORDING IN OUTER SEGMENTS OF RED AND GREEN RODS OF THE TOAD RETINA. Kenneth T. Brown and Dale G. Flaming*. Dept. Physiol., Sch. Med., Univ. Calif., San Francisco, CA 94143.

Amphibian green rods have been inaccessible to intracellular recording because of their sparse representation and very thin inner segments. We have now developed microelectrode techniques which permit systematic intracellular recording in the outer segments of toad rods, which are 5.0-7.5 µm in diameter. An electrode puller has been developed which reliably forms tips only 0.02-0.07 µm in diameter. These tips are also exceptionally short, hence have resistances of only 25-165 MΩ when filled with 5M K-acetate, and are stiff enough to be beveled by techniques reported previously. We have also devised a stepping hydraulic microdrive that advances by high speed steps featuring high acceleration, velocities up to 2 µm/msec, and negligible after-vibrations. Detection of a cell's membrane potential may be used to stop this microdrive on the same 1 µm step that carries the electrode into the cell, thus preventing cell damage by over-penetration. Using an isolated and inverted retina, contact of the electrode with the tip of a rod outer segment is visualized by infrared light and a high-resolution TV camera. At shallow depths containing only rod outer segments, several are often penetrated along a single low-angle electrode track. Large light responses have been obtained (up to 30 mV) and held for 1-2 hrs with no sign of deterioration. In agreement with previous work, the red rod thus far appears univariant, its responses being influenced only by summative interaction with other red rods. At short wavelengths near the λmax of its photopigment, the green rod gives a hyperpolarizing response similar to that of red rods. But the green rod is not univariant. It exhibits a hyperpolarizing input from another class of receptor tentatively identified as the red rod. Furthermore, at long wavelengths it shows a delayed and slow depolarization, probably due to inputs from single cones. These receptor interactions differ from those reported to date in other vertebrate photoreceptors, and are thus of special interest, especially since they occur in a short wavelength receptor that appears to participate in color vision. (Supported by NIH grant No. EY 00468).

1756 LARGE VISUAL RECEPTIVE FIELDS IN A POLYSENSORY AREA IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE. Charles J. Bruce*, Robert Desimone*, and Charles G. Gross. Dept. Psychol., Princeton Univ., Princeton, NJ 08540

Multi-unit and single unit activity was recorded from the dorsal bank of the superior temporal sulcus in immobilized macaques under N_2O. 70 multi-unit penetrations revealed an area responsive to visual, auditory and somesthetic stimuli which corresponds approximately to Jones and Burton's cytoarchitectonic Area T3 (JCN 168, 197). Of 79 isolated single neurons, 96% were responsive to visual stimuli, 55% to somesthetic and 29% to auditory; 44% were bimodal and 18% trimodal. The visual responses were binocular and the visual receptive fields huge: 75% extended into the monocular crescent of both eyes and 78% extended more than 45° into both upper and lower fields. Responsiveness to stimuli in the far periphery was usually similar to that at the fovea. Virtually all the somesthetic receptive fields were also large and bilateral.

Almost all units responded more to moving than flashed stimuli; size, orientation and direction of movement were usually unimportant but many cells were differentially sensitive to visual stimuli entering or leaving the field, approaching or withdrawing, and expanding or contracting. Some units had other specific visual trigger features.

This area receives an input from inferior temporal cortex and is reciprocally connected with area 7 (Jones and Powell, Brain 93, 793; Mesulam et al., Br. Res., in press); its units share some properties with both these areas.

1757 AN AUTORADIOGRAPHIC STUDY OF PRIMARY RETINAL PROJECTIONS IN GROUND SQUIRRELS. Laura L. Bruce* and Earl Kicliter, School of Basic Medical Sciences and Neural and Behavioral Biology Program, University of Illinois at Urbana-Champaign, IL 61801.

Retinal projections of 13-lined ground squirrels (Citellus tridecemlineatus) were determined with autoradiographic methods after unilateral intraocular injections of 50 µCi-1 mCi ^{3}H-proline and post-injection survivals of 24 hours to 10 days. The following targets were identified: Suprachiasmatic nucleus: SCN labeling is denser contralaterally. Accessory optic system: Contralateral medial, lateral and dorsal terminal nuclei receive retinal projections. Ventral lateral geniculate: LGv is bounded dorsally by the intergeniculate leaflet which receives a bilateral projection. Label in the remainder of LGv is denser contralaterally and is distributed in both internal and external sublayers of the external layer. Dorsal lateral geniculate: Three laminae are revealed in coronal sections. Laminae 1 and 3 receive a contralateral projection, while lamina 2 receives an ipsilateral projection. Pretectum: Of the three pretectal nuclei which receive retinal projections, only the olivary pretectal nucleus (OP) receives projections from both eyes. Both ipsilateral and contralateral projections to OP are divided into two segments. The posterior pretectal nucleus (PPN) lies posteromedial to OP and receives a diffuse contralateral projection. The retinal projection to the contralateral nucleus of the optic tract (NOT) begins dorsally and slightly posterior to OP; silver granules are arranged in dense clusters. Posteriorly, OP, PPN and NOT merge at the medial edge of the brachium of SC. Superior colliculus: In the contralateral SC, stratum zonale and stratum opticum (SO) are densely labeled; stratum griseum superficiale (SG) contains less label. The ipsilateral SC contains small puffs of grains distributed primarily in the lateral, anterior region of the SC, at the boundary of SG and SO. Medial geniculate nucleus: Diffuse but distinct labeling is observed in animals which receive high doses of ^{3}H-proline. Pulvinar: Silver grain distribution indicates bundles of axons of passage and a possible small projection. The present results correlate closely with previous investigations except for projections to SCN, MGN and pulvinar. Of these, that to MGN is intriguing since Kalil and Schneider (Brain Res. 100:690, 1975) observed a retinal projection to MGN after ablation of the SC and brachium of the inferior colliculus in neonate hamsters. The projection observed by Kalil and Schneider may thus represent only increased axonal termination in MGN rather than an entirely new projection. (Supported by USPHS Grant EY-01736 to E. K. and NSF SER 76-18255.)

1758 A COMPARISON OF SUPRAGRANULAR TO INFRAGRANULAR CONNECTIONS IN THE VISUAL CORTEX OF NORMAL AND VISUALLY DEPRIVED RATS. Albert B. Butler and John A. Jane. Dept of Neurosurgery, University of Virginia, Charlottesville, VA 22901

We have examined the connections between supragranular and infragranular layers of visual cortex in normal animals and animals who underwent neonatal bilateral eyelid suture. Thermal lesions were made through the intact skull in layers I, II, and the upper part of layer III in visual cortex of both normal and visually deprived adult rats. Transcardial perfusion fixation with aldehydes was performed on post-lesion days 2 or 3 for electron microscopy. 1 X 1 mm blocks of tissue extending through the depth of the cortex were taken from the lesioned area and embedded using routine electron microscopic techniques. The infragranular cortex of both groups was then examined using light and electron microscopes. The identification of specific laminae was determined by examining adjacent thick (1 µm) and thin sections. The thin section was first studied for areas of degeneration and the location of these areas determined by comparing these zones to the same areas on thick sections. In the normal animal, degenerating axon terminals are seen almost entirely within layer V and are found mainly on dendritic spines and less often on dendritic shafts. In the deprived animals, however, an apparent redistribution of degenerating axon terminals is seen with a relative decrease in the number of terminals ending on dendritic spines, with a relatively greater number ending on dendritic shafts and neuronal perikarya.

1759 EFFECTS OF VISUAL DEPRIVATION ON THE DEVELOPMENT OF VISUAL PATHWAYS IN THE GALAGO. V. A. Casagrande, D. Raczkowski*, and I. T. Diamond. Dept. Anat., Vanderbilt Univ., Nashville, TN 37232, Dept. Psych., Duke Univ. Durham, N. C. 27706.

We examined geniculate cell sizes and distribution of retinofugal and geniculocortical axons in two galagos raised for 1 year with a monocular lid closure. Measurements of cell size in the lateral geniculate nucleus showed that deprived cells in both the binocular and monocular segments were significantly smaller than cells innervated by the open eye. However, mean asymmetries between sizes of deprived and non-deprived cells were on the average considerably greater in the binocular segment (30%) than in the monocular segment (12.5%). These findings support results obtained in monocularly deprived cats and monkeys indicating that both binocular imbalance and deprivation per se affect cell growth (Von Noorden et al. Brain Res. 111: 277, 1976; Hickey et al. JCN 172: 265, 1977).

Comparisons of cell measures in individual layers indicated that changes in both absolute and relative cell size within the magnocellular layers were within the range of cell size changes seen in the parvocellular layers.

Changes in retinal and geniculo-cortical properties were investigated following injections of ^{3}H proline into either the open or sutured eye in the deprived galagos and into one eye in each of the normal galagos. No evidence was found for changes in the distribution of retinal axons to geniculate nucleus or to the superior colliculus. In both normal and deprived galagos label in the superior colliculus appeared continuous contralateral to the eye injection and patchy ipsilateral to the eye injection. No obvious differences in the width of these ipsilateral patches nor discontinuities of the contralateral distribution could be seen between animals.

In contrast with the retino-tectal projections, transsynaptically transported label to striate cortex showed several changes following deprivation. Injections into the sutured eye resulted in distinct patches with the binocular portion of layer IV of both the ipsilateral and contralateral striate cortex. These patches were much more pronounced than those seen in the normal galago. Injections into the non-deprived eye revealed continuous label within layer IV of striate cortex in both hemispheres. These results are consistent with earlier reports in cat and monkey showing rearrangement of geniculo-cortical axons following monocular deprivation.
(Supported by Grants EYO-01778 and MH-4849.)

1760 RELATIONSHIP BETWEEN INTEROCULAR ALIGNMENT AND BINOCULARITY OF STRIATE CORTICAL NEURONS IN SIAMESE CATS. Y.M. Chino* and M.S. Shansky*. Division of Visual Science, Illinois College of Optometry, Chicago, IL 60616. W.J. Pizzi. Dept. of Psychology, Northeastern Illinois University, Chicago, IL 60625. (SPON: T.S. Brown)

Responses of cortical cells in the striate cortex of Siamese cats were recorded extracellularly, under nitrous oxide/oxygen anaesthesia and with tungsten-in-glass-microelectrodes. Binocular activation of each unit was assessed by determining its ocular dominance group (Hubel and Wiesel, 1962). In addition, responses were recorded from cortical cells in normal cats for the purposes of comparison. Although the dominance distributions for the Siamese strongly favors contralateral innervation, there are a higher percentage of binocularly-activated cells than has previously been reported, and many of these had receptive fields within 8° of the area centralis. Furthermore, the data suggests an inverse relationship between the percentage of binocularly excited cells and the extent of convergent misalignment exhibited by individual Siamese cats.

1761 PULVINAR LESIONS IN MONKEYS PRODUCE ABNORMAL SCANNING OF COMPLEX VISUAL ARRAYS. Carol A. Christensen* and Leslie G. Ungerleider. Psychology Department, Vassar College, Poughkeepsie, N.Y. 12601 and Laboratory of Neuropsychology, NIMH, Bethesda, MD. 20014.

Visual orientation and habituation to a complex stimulus array were studied in rhesus monkeys with bilateral pulvinar lesions (N=4) and in normal control monkeys (N=4). The stimulus array consisted of 16 geometric figures; it was presented for 5-sec trials, 40 trials per day, on two consecutive days. On the first and last 10 trials of each day's session, all of the figures appeared as white stimuli on a black background; on trials 11-30, one of the figures within the array was colored red. Using a corneal reflection technique, visual orientation and habituation to this novel stimulus were recorded by video taping the monkey's eye movements as he scanned the stimulus array. Subsequent to behavioral testing, the video tape records were analyzed to determine the monkey's point of fixation at 200-msec intervals for each trial.

The results revealed that pulvinar lesions did not alter either visual orientation or habituation to a novel stimulus. Both normal monkeys and monkeys with pulvinar lesions shifted their gaze towards the figure to which color was added; habituation to the novel stimulus occurred at varying rates for individual animals, but did not differ between normal and operated monkeys. However, pulvinar lesions did affect visual behavior in two significant ways. First, unlike normal monkeys, monkeys with pulvinar lesions displayed a paucity of saccades off the stimulus array; that is, they appeared "visually captured" by the stimuli. Second, the visual fixations of monkeys with pulvinar lesions were abnormally prolonged, on the average 300 msec longer than those of normal monkeys. Prolonged fixations were observed both when the operated monkeys fixated the white geometric figures and when they fixated the novel stimulus. In contrast to these abnormalities in visual scanning, damage to the pulvinar did not produce any detectable oculomotor impairment; results of neurological tests indicated that optokinetic nystagmus, convergence, vertical and horizontal saccades, and visual pursuit were all normal in monkeys with pulvinar lesions.

We have previously reported that monkeys with pulvinar lesions demonstrate visual capture and prolonged fixations during visual discrimination learning (ARVO Meeting, 1977). The present results extend these findings: scanning abnormalities produced by pulvinar damage occur not only during discrimination training but also during spontaneous viewing of a complex stimulus array.

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1762 NASO-TEMPORAL DIVISION OF RETINOTHALAMIC PATHWAY IN NORMAL AND SIAMESE CATS. Michael Lee Cooper* and John Pettigrew* (SPON: Masakazu Konishi). Div. of Biology, Calif. Inst. of Technology, Pasadena CA 91125.

The naso-temporal division of the retinothalamic pathway was studied in normal and Siamese cats. One lateral geniculate nucleus (LGN) of each cat was filled with horseradish peroxidase (HRP); two days later both retinae were removed and reacted with benzidine in order to visualize HRP retrogradely transported to the ganglion cell bodies. Ganglion cell density maps of the temporal retinae were then constructed from retinal whole-mounts.

The normal cats showed clear vertical decussation lines passing through the area centralis (AC). The ipsilateral decussation was sharp and passed through the center of the AC; the contralateral decussation was somewhat less sharp, with scattered cells extending a few degrees into the temporal retina (as previously reported by Stone).

In the Siamese cats there was no sharp vertical decussation line between areas projecting ipsilaterally or contralaterally; rather, there was overlap in the temporal retina between cell populations projecting to each hemisphere. Thus, there was no region of exclusive contralateral misprojection extending 20° temporally from the vertical meridian. Contour maps of the percentages of cells projecting ipsilaterally showed a smooth gradient-like decrease in the percentage of cells misprojecting contralaterally as one proceeded temporally. In other words, there was no special region of unilateral projection; all parts of the temporal retina projected bilaterally. In addition, the ganglion cell isodensity lines were elongated along the horizontal visual streak in both ipsilateral and contralateral retinae.

Cell-size measurements were made at equivalent points in the ipsilateral and contralateral retinae. In a field approximately 20° along the horizontal meridian in the temporal retina, about 2% of the large cells projected ipsilaterally, while about 96% of these large cells projected contralaterally. At this location, about 30% of the total retinothalamic population sent axons ipsilaterally, indicating that the large (presumably Y-type) cells are more affected by the Siamese defect than the ganglion cell population as a whole.

A differential effect of the Siamese defect as a function of cell class was supported by anterograde transport of tritiated proline from the eye. This revealed labeling of the ipsilateral C1-lamina over a wider range of eccentricities than the ipsilateral A1-lamina, suggesting that W-type cells maintain their normal projections in regions in which cells destined for the A1-lamina tend to misproject.

1763 TECTAL PROJECTION OF DISPLACED RETINAL GANGLION CELLS IN THE CHICK EMBRYO. James E. Crandall*, Marieta B. Heaton and Idania M. Alvarez* (SPON: Christiana M. Leonard). Dept. Neuroscience, Coll. Med., Univ. Fla., Gainesville, FL. 32610.

Displaced ganglion cells (DGC's) were first described in the avian retina by Dogiel in 1888. These cells resemble typical retinal ganglion cells in most respects, but rather than residing within the ganglion cell layer, they occupy a seemingly aberrant position, just above the boundary of the inner plexiform and inner nuclear layer. These cells may have a special functional significance since they appear to be the only retinal ganglion cells receiving a direct centrifugal projection (Maturana and Frenk, Science,150:359, 1965), but it is not known whether their field of innervation differs from those of the normal ganglion cells. We studied the projections of the DGC in the chick embryo retina by making discrete (.1-.4 µl) tectal injections of horseradish peroxidase (HRP) and incubating the tissue in diaminobenzidene followed by paraffin embedding and sectioning. These studies revealed the following: Approximately 7,900 DGC's are labeled in the retina of 18 day chick embryos following tectal injections. These labeled cells are between 10-15 µ in diameter, about twice the size of orthotopic ganglion cells. Since unlabeled DGC's could not be distinguished from giant amacrine cells, it was not possible to determine what proportion of the total DGC population projects to the tectum. Discrete tectal injections as early as 10.5 days of incubation revealed labeled DGC's. Labeled DGC's have been previously seen following HRP tectal injections in rats (Bunt, et. al., Brain Res. 73:215, 1974). However, experiments in chick hatchlings (LaVail and LaVail, JCN 157:215, 1974) and adult pigeons (Karten, ARVO, 1977) have suggested that the major projection is extratectal in the avian. The DGC tectal projection in chick embryos may represent collaterals of fibers terminating in extratectal sites or may originate from a separate DGC population.

1764 TOPOGRAPHIC PROJECTIONS OF RETINA AND OPTIC TECTUM UPON THE AVIAN VENTRAL LATERAL GENICULATE NUCLEUS. W.J. Crossland. Dept. Anat., Sch. Med., Wayne State Univ., Detroit, MI 48201.

The avian ventral lateral geniculate nucleus (GLv) located in the ventral diencephalon consists of two laminae oriented parallel to the optic tract. The outer lamina, proximal to the tract, contains loosely scattered cell bodies in a matrix of neuropil. The inner lamina, distal to the tract consists of a layer of densely aggregated cell bodies. Golgi studies have revealed that these cells project toward the optic tract within the outer lamina. By making small injections of 3H-proline into the retina and using the autoradiographic tracing method, we have found, 1) that the retinal input is confined to the outer lamina (neuropil) with a peak of terminal density near the junction of the inner and outer laminae, 2) that the Glv is retinotopically organized: anterior retina projects upon rostromedial Glv, posterior retina upon caudolateral GLv, superior retina upon caudomedial GLv and inferior retina upon rostrolateral GLv.

Stereotaxic injections of tritiated amino acids made into the optic tectum revealed a topographic projection of the tectum upon the GLv. Analysis of the retinogeniculate and retinotectal projection indicates that the tectogeniculate map is in register with the retinogeniculate map. In contrast with the retinal projection, the distribution of tectal terminals is relatively sparse near the inner cell lamina. Thus there is some segregation of input to different strata of the outer lamina. (Supported by NIH Grant EY-01796.)

1765 QUANTITATIVE STUDIES OF IN VIVO FROG RETINAL GANGLION CELL RESPONSES TO CHROMATIC AND SPATIAL STIMULI. Peter F. Cummings*, Gerald F. Hungerford* and Richard L. Binggeli. Dept. Anat., Sch. Med., USC, Los Angeles, CA 90033.

Frog retinal ganglion cells (GC) were studied using an in vivo eyecup preparation. Adult, pithed Rana pipiens obtained from Northern Mexico were used. Extracellular recordings were made from either GC bodies or axons using sodium-acetate filled glass micropipiettes at a depth of 20 to 150μ from the retinal surface. The preparation was studied with a constant background illumination of about 1 X 10^9 photons/mm^2/sec. All stimulus presentations were centered in the apparently active receptive field of the GC, and were repeated 10 times. The stimulus duration was 500 ms. on and 1500 ms. off. 21 different stimuli were presented to each cell. Only cells which were held for 30 min. and presented with all 21 stimuli were included in the data.

The intensity of all stimuli was adjusted with neutral density filters to approximately 1.5 X 10^{12} photons/mm^2/sec. A monochrometer was used to set the light wavelength of each of the spatial stimuli at 432 nm, 502 nm, and 572 nm. Seven spatial stimuli were used which included light spots (2.5°, 5°, & 22°), light annuli (2.5°x5°, 2.5°x22°, & 5°x22°), and dark annuli (2.5°x5°).

Ganglion cell responses, recorded on magnetic tape, were processed and compiled into post-stimulus-time-histograms from the 10 replications. These were used to generate statistics quantitatively descriptive of the response. These statistics were presented as distributions for all cells in all 21 stimulus conditions. Preliminary analysis of this data shows wide variation in response from cell to cell (0-13 spikes per on period). Cells showing spontaneous activity, adaption, and chromatic & stimulus size differentiation were observed. In general, the mean number of spikes during the on period of the stimulus presentations was low (1-3) and the variation between means was small. The mean value of the on response to the 5° spot tended to be larger than the response to the 2.5° or 22° light spot.

1766 SPATIO-TEMPORAL RECEPTIVE FIELD STRUCTURE OF RETINAL NEURONS IN THE CATFISH. Wes Davis* and Syozo Yasui* (SPON: K. I. Naka). Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Various types of receptive fields, spatio-temporal response patterns of vertebrate retinal neurons, have been described but no systematic effort has been made to correlate underlying morphology to the functional receptive fields. We have recorded from and dye-injected and recovered in flat mount more than 200 catfish (Ictalurus punctatus) retinal neurons. We have defined their spatial organization by stimulating the retina with a moving white-noise spatial grating and cross-correlated the resulting responses recorded intracellularly against the input. The first order correlation is the best linear approximation of the field organization revealed by a bar of light moving across the field at a constant speed. The use of a white-noise spatial grating has many advantages over the much simpler method of moving a single bar of light.

Morphological and functional correlation was made by superposing the images of neurons on the cross-correlograms.

We have found 1) the cell bodies and axons of the external horizontal cells formed a monophasic receptive field although the latter's field was much wider, 2) all bipolar cells (as defined morphologically) had an antagonistic center-surround field and both the on- and off-center variants had almost identical spatial dynamics, 3) the sustained amacrine cells showed a complex field organization, and 4) some ganglion cells formed antagonistic center-surround fields whereas others formed a monophasic field. The size of the field of the proximal neurons was often equal to or smaller than the size of their dendritic field.

We will discuss how morphological subclasses of each neuron type form different functional fields.

1767 COMBINATION OF MONOCULAR AND DIRECTIONAL DEPRIVATION IN THE KITTEN VISUAL CORTEX. Nigel W. Daw, Nancy Berman and Michael Ariel.* Department of Physiology and Biophysics, Washington University Medical School, St. Louis, MO 63110.

Six kittens were reared with both monocular (in this case reverse suture) and directional deprivation to see if the effects of different types of visual deprivation are independent of each other. The kittens were placed in a drum rotating in one direction with one eye open from 2 1/2 to 5 weeks, followed by a drum rotating in the other direction with the other eye open from 5 to 12 weeks. Recordings were then made in the visual cortex. The percentage of cells driven by one eye or the other and the overall percentage of unidirectional cells was found to be a function of the reverse suture. Among unidirectional cells, the proportion of cells preferring one direction versus the other was a function of the directional deprivation. The results of reverse suture plus directional deprivation were the sum of the results of these two deprivations taken alone. However reverse suture affected the directional properties of the cells, just as directional deprivation has been shown to affect the ocular dominance histogram.

1768 EXTRACELLULAR POTASSIUM ACTIVITY IN THE MUDPUPPY RETINA; ITS RELATIONSHIP TO THE B-WAVE OF THE ERG. Evan Dick* and Robert F. Miller. Division of Neurobiology, Dept. Physiol., SUNYAB, Buffalo, N.Y. 14214.

In the perfused retina-eyecup preparation of the mudpuppy, double-barreled microelectrodes were used to simultaneously record changes in extracellular potassium (K^+_o) activity and the ERG evoked by diffuse light stimuli.

In the distal retina, near the R-membrane, the K^+_o reponse consists of an immediate, gradual decrease to light onset and a gradual return to the dark baseline at offset. This response is unaffected by a brief application of 2mM Co^{++}, consistent with its generation by photoreceptors. Near the level of the maximum inverted b-wave (70% retinal depth) the K^+_o response to light onset consists of a transient increase followed by a slow decrease and return to baseline at light offset. The K^+_o increase at light onset is abolished by Co^{++} indicating that it results from post receptor neuronal activity. In the proximal retina the K^+_o response consists of a slow increase to both light onset and offset.

We studied the effects of a number of pharmacological agents (6% ethanol, 2mM GABA, 2mM glycine, 0.8mM alpha-aminopimelic acid, and combinations of these) in order to assess whether the K^+_o increase to light onset observed in the distal vs. proximal retina results from activity of a single class of cells. Our data clearly show that different populations of neurons are associated with proximal and distal K^+_o activity.

Application of agents which increase b-wave amplitude (ethanol, ethanol plus GABA) also causes an increase in the distal K^+_o increase seen at light onset. These same agents either have no effect on or, in some cases, diminish the light evoked K^+_o response in the proximal retina. Similarly, other agents (GABA, alpha-aminopimelic acid) which cause a marked reduction in the proximal K^+_o response have little or no effect on either the b-wave or the distal K^+_o increase.

Our findings suggest that light evoked increases in K^+_o activity in the proximal and distal retina can be pharmacologically separated and must be generated by more than one population of cells. We further suggest that the K^+_o increase seen in the distal retina in response to light may be associated through Muller cell depolarization with the site of b-wave initiation. Intracellular recordings from neurons suggests that depolarizing bipolars may be intimately linked to b-wave generation.

1769 RECEPTIVE FIELD CHARACTERISTICS OF VISUAL CELLS IN THE SUPERIOR COLLICULUS OF THE GOLDEN HAMSTER. James Dixon* and Barry E. Stein (Spon: R.J. Krieg). Dept. Physiology, Medical College of Virginia, Richmond, VA 23298.

Neurons activated by moving and/or stationary visual stimuli were studied in all strata of the hamster superior colliculus (SC). Animals were anesthetized with urethane and, in selected experiments, paralyzed with gallamine triethiodide and artificially respired. Neuronal recordings (n=264) were conducted using tungsten microelectrodes. Visual receptive fields (RFs) were mapped on a translucent hemisphere positioned 28 cm in front of the animal. Moving and stationary light stimuli were presented on this hemisphere and quantitative evaluation of RF properties was facilitated by the use of a galvanometer-driven mirror system with which precise changes in stimulus parameters were accomplished.

The upper laminae of the SC were exclusively visual, whereas somatic, acoustic, nociceptive and multimodal cells were encountered in the intermediate and deeper layers. The frequency of visual cells decreased with depth in the SC and their RFs increased in size. However, upper and lower layer cells had many characteristics in common.

For most cells, RFs could not be subdivided on the basis of responses to stationary pulsed light. However, systematic changes in the size of a light bar which was moved through the RF revealed a nonhomogeneous internal organization. Optimal responses were initiated in 94% of the cells when the stimulus was 35% or less of the RF extent. Increasing stimulus size byyond the optimum resulted in progressive and significant decrements in impulse frequency. Further, but less dramatic, decrements were initiated in 61% of the cells when stimuli extended beyond the RF borders. Direction of movement was a critical variable in 56% of the cells. A bar of light was moved through the RF in 8 directions (4 axes) and directional selectivity was defined as a 2:1 ratio between the discharge rates elicited by opposing movements in the same axis. The one direction most frequently preferred was temporal-nasal. Directional selectivity decreased as velocity was shifted from the optimum (most frequently 5-10°/sec or 40-50°/sec).

The laminar organization of sensory representation in the hamster and cat SC are similar. Furthermore, visual SC cells of both species are similar in their preferences for moving stimuli. However, while the optimum velocities are the same for the two species, their directional preferences are 180° out of phase. At present we know of no ecological requirements which would favor this difference in neuronal specialization.

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1770 DIVERGENT PROCESSING OF VISUAL INFORMATION IN AREAS 17 AND 18 OF THE RHESUS MONKEY. Bruce Dow, Division of Neurobiology, Department of Physiology, SUNY/BUFFALO, Amherst, N.Y. 14226, and NATIONAL EYE INSTITUTE, Bethesda, Md. 20014.

Moving bars of light have been used to test the responses of foveal cortical cells in areas 17 and 18 of anesthetized rhesus monkeys. Tuning curves obtained with such stimuli have been converted into points on a scatter plot, with "directionality" as the ordinate and "angular selectivity" as the abscissa. This display permits comparison of cells with regard to location in two-dimensional specificity space. Distinct clusters of direction cells and orientation cells are evident in the scatter plot. Each cluster contains cells from both area 17 and area 18. Direction cells are sensitive to velocity as well as direction of movement. Orientation cells require elongated as well as properly oriented stimuli. Among 268 cells (179 in area 17, 89 in area 18) 40% are orientation cells and 15% are direction cells. The proportions are the same in areas 17 and 18.

About 26% of the cells in each area are color cells. The great majority of color cells fall outside of the orientation and direction clusters. In area 17, only 6% of the cells are both orientation- and color-specific, and even fewer (1%) are both direction- and color-specific. In area 18, 1% are orientation- and color-specific, and 1% are direction- and color-specific.

The laminar distribution of 198 cells (119 in area 17, 79 in area 18) has been determined. Direction cells tend to be most common in layer 4A (just above the granule cells) in each area, whereas color cells are rarely found in layer 4A. Orientation cells are about equally common in all layers of both areas.

The data suggest the existence of neuronal mechanisms in areas 17 and 18 for refining and separating different kinds of visual specificity (line orientation, movement direction, color).

1771 ELECTRON MICROSCOPIC EVIDENCE OF A DORSOTEMPORAL TO VENTRONASAL GRADIENT IN FIBER SIZE WITHIN PIGEON OPTIC NERVE. Thomas A. Duff and Grayson Scott*. Depts. of Neurosurg. and Anatomy, Univ. of Wisc., Madison, WI 53706.

Using a uniform sampling method, electron microscopic examination of pigeon optic nerve revealed a dorsotemporal to ventronasal gradient in mean fiber size. The ventronasal portion of the nerve contained large myelinated axons possessing a mean cross sectional area, including myelin sheath, of 0.89 μ^2, S.D.=0.98. In the dorsotemporal portion small myelinated axons (mean area=0.43 μ^2, S.D.=0.35) were predominant. Also unique to this region were scattered clusters of fine, unmyelinated fibers. Axons located in the area between these two regions possessed a mean area of 0.72 μ^2, S.D.=0.78. This temporal to nasal gradient was found to be a constant feature throughout the length of the nerve.

The total number of fibers within the nerve, excluding those comprising the unmyelinated clusters, was calculated to be approximately 2.5 x 10^6. The overall distribution in fiber size was found to be unimodal, with the peak at 0.24 - 0.40 μ^2, and overall mean fiber area = 0.69 μ^2.

Although other investigators have reported a homogeneous spectrum in fiber size throughout pigeon optic nerve, the findings of the present study may be more compatable with anatomical and physiological features of pigeon retinotectal organization. These features include visual field acuity gradients, ganglion cell population pattern, difference in length of tectal afferents arising from ventronasal vs. dorsotemporal retina, and density fields of tectal afferent terminations.

1772 OCULAR ALIGNMENT AND VISUAL FIELD ABNORMALITIES FOLLOWING NEONATAL SECTION OF THE POSTERIOR CORPUS CALLOSUM IN THE CAT. Andrea J. Elberger* (SPON: J. M. Sprague). SUNY at Stony Brook, Stony Brook, New York, 11794.

In young cats, the posterior portion of the corpus callosum was sectioned 13-41 days after birth. The animals' eyes were photographed at weekly intervals for 6 months using the pupil-reflex method. From the corneal reflection evident in the photographs the degree of alignment for the optical axes of each cat was estimated (Sherman, 1972). The 17 experimental cats all showed a clear, permanent divergent strabismus, as compared to 6 normal cats.

After 7 months the limits of the visual field were determined for both groups of cats using a perimetry technique similar to that of Sprague and Meikle (1965). With both eyes open experimental and normal cats could see to 90° on either side of the vertical midline. With one eye open normal cats responded from 90° ipsilateral to 45° past the vertical midline into the contralateral visual field, while with either eye the experimental cats saw no more than 2-3° past the vertical midline. That loss of visual responsiveness is within the contralateral region of the normally binocular zone.

Three normal adults, 9, 13 and 20 months old, were photographed for at least 5 consecutive weeks using the pupil-reflex method. These results were identical to those of normal cats 5-7 months old. Then, the adults received section of the posterior corpus callosum. Photographs of the pupil reflex were taken again for at least 5 consecutive weeks after surgery. These results were identical to those of pre-surgery. In addition, adults were tested on visual field perimetry 3-4 months after surgery. These results were identical to those of normal young cats.

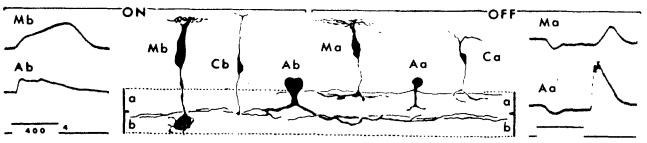
The present results indicate that the normal process of ocular alignment in young cats may be disrupted by early posterior callosal section. It is not known whether the observed abnormalities result from arrested development, or disruption of intrinsically determined ocular alignment. In either case, there is a limited time period for callosal surgery to produce abnormal interocular alignment and abnormal visual field perimetry.

Preliminary results using both photographs of the pupil-reflex method and projection of optic discs indicate that in paralyzed experimental cats there is no evidence of an abnormality in eye position. This suggests that the divergence characteristic of the waking state reflects some dynamic imbalance of ocular alignment. (Supported by NIMH Grant #25643).

1773 NEURONAL ARCHITECTURE OF ON AND OFF PATHWAYS IN CARP RETINA. E. Famiglietti, A. Kaneko* and M. Tachibana*. Departments of Physiology, Wash. Univ. Sch. Med., St. Louis, MO and Keio Univ. Sch. Med., Tokyo, Japan.

It has been demonstrated that the inner plexiform layer (IPL) of cat retina can be divided into two sublaminae (a & b), containing the terminals of flat and invaginating cone bipolars, respectively [1]. In that study, the majority of retinal ganglion cells could be subdivided into types a and b, according to the sublamina in which their dendritic trees were confined. Subsequently, intracellular staining was used to confirm the hypothesis that sublamina a contains the synapses for OFF responses and sublamina b for ON responses.[2]. We find that carp retina is similarly organized.

Procion yellow-stained carp bipolars (25) could be divided into large (M) and small (C) varieties (fig.). These in turn could be subdivided into types a and b, according to the destination of terminals in the IPL. All type a cells were hyperpolarizing (OFF) and all type b cells were depolarizing (ON). All bipolars showed a maximal response to red in the receptive field center. Many had color-opponent, green-dominant surrounds.



Procion-stained amacrine and ganglion cells (25) were also restricted in their branching to sublamina a or b, when their dominant (sustained) response was OFF or ON, respectively (fig.). ON-OFF cells branched diffusely in a & b. Cells with branches in stratum (S)3 showed complex chromatic responses with prominent short wavelength effects. The terminals of color-coded Ma and Mb bipolars in S1&2 and S4&5, respectively, lie in appropriate position to determine sustained amacrine responses of similar polarity, as well as center-opponent responses of red-green, double opponent ganglion cells. These data indicate that bisublaminar organization of the IPL, separating ON and OFF mechanisms, is a general principle of retinal organization in vertebrates.

1. Famiglietti and Kolb, Science, 194,193,1976.
2. Nelson, Famiglietti and Kolb, in press.

1774 VISUAL AND EXTRA-VISUAL RESPONSES OF SINGLE NEURONS IN THE PULVINAR AND LATERALIS POSTERIOR NUCLEI OF THE CAT. Steven E. Fish* and Leo M. Chalupa* (SPON: J. S. Robinson). Dept. of Psychology, University of California, Davis, CA 95616.

Extra-cellular single cell recordings were carried out from the pulvinar and lateralis posterior nuclei of cats maintained on a mixture of nitrous oxide and oxygen. Neurons were tested for responses to visual, auditory, and somatosensory stimulations and for responsive cells, receptive field characteristics were investigated.

Approximately 55% of all cells isolated in the pulvinar-LP complex did not respond reliably to sensory stimulations, although some of these could be diffusely activated by one or more modalities. About 30% of the cells tested yielded secure responses to visual stimulation. Typically these had large receptive fields (about 20 degrees in diameter), and generally did not show directional selectivity or orientation specificity. A small proportion of the visual cells exhibited complex response properties, including speed preferences, directional selectivity and/or orientation specificity. The remaining 15% of the cells tested responded to auditory and/or somatosensory stimulation. Multimodal cells were grouped in the ventral portion of the pulvinar-LP complex. This region receives a direct projection from the deeper layers of the superior colliculus. Some of the multimodal cells showed a clear correspondence of visual and extra-visual receptive fields.

1775 THE ACCESSORY OPTIC SYSTEM AND OPTOKINETIC NYSTAGMUS. Katherine Fite, Tony Reiner*, and Stephen Hunt*. Dept. Psychol. U. Mass. Amherst, 01003 and Dept. Anat., SUNY at Stony Brook, 11794.

In nearly all vertebrates, the accessory optic nuclei of the midbrain receive a direct retinal projection. In pigeons, this projection originates from large, displaced ganglion cells located primarily in the peripheral retina whose axons terminate upon the nucleus of the basal optic root (nBOR)--a component of the accessory optic nuclei (Karten, Fite & Brecha, PNAS, 1977). NBOR, in turn, projects monosynaptically to the vestibulocerebellum (uvula, folia IX) and to the oculomotor nuclear complex (Brauth & Karten, Exp. Brain Res., 1977). It has been suggested that some aspects of oculomotor function may be mediated via these pathways, and Lazar (1973) has shown that lesions of the accessory optic nuclei in frogs completely eliminates optokinetic nystagmus (OKN).

The present study demonstrates that stereotaxically placed electrolytic lesions which damage portions of the nBOR of pigeons (Columba livia) and turtle (Chrysemys picta picta) are correlated with either a decrease or a loss of optomotor response when compared with pre-lesion response functions obtained on individual subjects. Using a conventional optokinetic stimulator, the average frequency of head movements/minute were measured as a function of pattern velocity over a range of 5-80 degrees/sec. for each subject and were obtained under both binocular and monocular viewing conditions for clockwise and counterclockwise directions of pattern movement. Following several weeks of post-lesion behavioral testing, both eyes of each subject were injected with 100 μC of (^{3}H) proline. One to four days later, subjects were anesthetized, perfused and the brains subsequently processed for autoradiography. This procedure enabled both a detailed reconstruction of lesion topography and the accurate assessment of remaining (intact) post-lesion optic projections to the accessory optic nuclei, as well as to the other midbrain retinal targets (pretectal complex, optic tectum).

In both pigeon and turtle, the extent of post-lesion change in OKN was correlated with the size and extent of damage to nBOR and to the degree of bilateral involvement. In several instances, lesions which destroyed a large portion of nBOR completely eliminated any measureable optomotor responses from the contralateral eye when tested monocularly with both directions of pattern movement. Thus, nBOR appears to mediate at least some portion of optomotor response in these two nonmammalian species.

1776 RESPONSES OF SINGLE UNITS IN VISUALLY ACTIVATED THALAMIC - PRETECTAL NUCLEI OF THE TELEOST, MICROPTERUS SALMOIDES. Michael J. Friedlander* (SPON: C.L. Prosser). Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Responses of more than 200 diencephalic - pretectal units were investigated. All animals were optically corrected before recording. Recording sites were established by reconstruction of electrode tracks.

Penetrations at increasing depths within 300 microns of the midline revealed: 1) A dorsomedial thalamic region. Units with small (<5°) and large (>20°) receptive fields were found. Latency distribution histograms of responses to optic nerve shock were unimodal with a peak at 7 msec., suggestive of direct retinal input. 2) An intermediate ventromedial thalamic zone. It contained multimodal cells responding to tactile or lateral line input as well as visual stimuli. Most of these cells had longer latencies to optic nerve shock (>10 msec.). 3) A deep ventral thalamic - hypothalamic region. The cells in this region had wide fields (>20°). Latency to optic nerve shock was >10 msec.

In addition to visual input, cells of areas 2 and 3 (above) received descending input from ipsilateral telencephalon, primarily from dorsolateral areas. Latencies to telencephalic shock were 4 to 18 msec., suggesting both monosynaptic and polysynaptic pathways. In some cells, rebound effects lasting up to several hundred msec. were seen after telencephalic stimulation. When visual stimuli were paired with telencephalic shocks at different intervals, an early inhibition followed by facilitation resulted.

Lateral to the medial thalamic area in the central pretectal region, are cells with small to medium fields (3°-11°). These cells showed a high degree of directional selectivity and preference for moving targets. A bimodal latency distribution to optic nerve shock was seen with peaks at 8 and 12 msec. Some of these cells were activated by tectal stimulation.

More laterally, units in nucleus rotundus had a wide range of receptive field sizes (8°-35°). Most of these cells were driven by ipsilateral tectal shock. A small number of these cells also gave an antidromic spike upon tectal stimulation, demonstrating reciprocal connections. Latencies to optic nerve shock were always >13 msec. This area did not show activation from telencephalic stimulation.

1777 THE EFFECT OF RELATIVE MOTION ON DIRECTIONALLY SPECIFIC PIGEON TECTAL UNITS. B. J. Frost and S. C. P. Wong, Dept. Psych., Queen's Univ., Kingston, Ont., Canada, K7L 3N6.

A large body of psychophysical literature indicates that the context in which a stimulus occurs can exert a dramatic influence on its appearance. This has been demonstrated for dimensions such as brightness, hue, line orientation and motion. Experiments on motion perception have suggested that rather than absolute retinal image motion, movement of one pattern relative to another (whether stationary or moving) is the prime determinant of perceived motion. Although stimuli in the real world seldom occur in isolation most single unit studies usually manipulate parameters of a single stimulus. We have varied the motion characteristics of surrounding background stimulus patterns and have found that they differentially modify the response to moving test stimuli presented to directionally specific pigeon tectal units. The motion characteristics of 130 cells from all laminae were studied using standard recording techniques and a dual channel optical system which permitted the independent manipulation of the motion characteristics of the two stimuli. Test stimuli were presented through one channel while moving background patterns of random dots, grating and noise patterns were presented through the other. Eighty-five percent of directionally specific neurons were completely inhibited by background patterns moved in the same direction (in-phase) as the stimulus. With the background moving in the opposite direction (anti-phase) there was no inhibition and in many cases their response was facilitated. Movement of the background pattern alone elicited no response from these units. However, movement of the test stimulus across a stationary background produced PSTH's similar to those produced by the test stimulus alone. For the majority of these cells different background patterns produced the same results although a few required specific patterns before they exhibited the effect. It might be argued that the results of these experiments could be produced by cells that are both direction and size specific. However, facilitation by backgrounds moving in anti-phase argues against such an interpretation, and suggests that relative motion rather than size is the important variable. Furthermore, in some cases velocity tuning curves appear to be sharpened by backgrounds moving in anti-phase compared with tuning curves obtained with a solitary test stimulus.

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1778 RECEPTIVE FIELD PROPERTIES OF RETINAL GANGLION CELLS IN TURTLE. J.E. Fulbrook* and A.M. Granda. Inst. for Neuroscience, Univ. of Delaware, Newark, DE 19711.

Single unit receptive fields were investigated in ganglion cell axons to moving or flashing colored light spots. Four cell types were distinguished: on, off, on-off and sustained. All of the cells were sensitive to moving stimuli at about 9^{o} sec^{-1}; many were directionally selective. Receptive fields were most sensitive to red light. In some cases the fields also showed sensitivity to green and blue light matched for equal quanta with red. Under dark-adapted conditions, receptive fields to red light were 3^{o} - 6^{o} in diameter. Receptive fields for equated green and blue lights were larger, 4^{o} - 10^{o} in diameter for the same cell. With light-adaptation, red receptive fields were little affected, but most green and blue fields disappeared. Plotted fields were often surrounded by an inhibitory annulus. In directionally-selective fields, however, the surround was asymmetric with a strong inhibitory region just prior to the receptive field in the preferred direction. A few cells were exceptionally sensitive to high-velocity scans, firing optimally to spots moving in excess of 180^{o} sec^{-1}.

1779 ANALYSIS OF SIMPLE AND COMPLEX DIRECTIONALLY SELECTIVE RECEPTIVE FIELDS IN AREA 17 OF THE CAT WITH PAIRS OF SEQUENCED LIGHT FLASHES. Leo Ganz and Ralph Felder. Dept. of Psychol., Stanford U., Stanford, CA 94305.

The interaction between ON-region activation and OFF-region activation has been studied in some 110 neurons recorded from 55 cats. Directionally selective neurons with simple receptive fields typically show ON- OFF-interactions reminiscent of the center-surround antagonism found in retinal ganglion cells and lateral geniculate nucleus neurons. For example, we have found that turning off a stimulus in an ON-region has the effect of inhibiting a subsequent OFF-response in an adjoining OFF-region in neurons with simple receptive fields and sustained temporal properties. This inhibition occurs even in stimulus sequences which are in that neuron's preferred direction of motion. We have found this inhibition to be strongest for stimulus asynchronies of 100 msec.

Directionally selective complex neurons typically show ON-Off-interactions which are sequence selective and facilitatory. For example, an ON-ON sequence in an ON-region and in the preferred direction exerts a very powerful facilitatory effect on the turning off of a stimulus in an adjoining OFF-region. This facilitatory effect is not present if the ON-ON sequence is presented in a non-preferred direction of motion. This mutual facilitation represents a further, decidedly non-linear response of complex cells. Our data suggests that this facilitatory interaction between ON-directionally selective regions and OFF-directionally selective regions can provide a mechanism for width selectivity as well as for selectivity to black-on-grey vs. white-on-grey moving objects.

We have also analyzed some of the temporal properties of these directionally selective facilitatory interactions between ON- and OFF-regions of complex cells. Strong effects (5-10 fold facilitations) can still be obtained with 700 msec between induction and test.

Supported by NIH Grant EY-01241-03 to L.G.

1780 BINOCULAR SPACE-TIME INTERACTIONS IN CAT VISUAL CORTEX. Jill C. Gardner * and Max Cynader . Dept. Psych., Dalhousie U., Halifax, N.S. B3H 4J1, Canada.

Units in ocular dominance groups 2,3,5 and 6 of Hubel and Wiesel respond with different strength to stimulation of each eye. We have found that a difference in response strength frequently corresponds with a difference in response latency, and that the latency through the stronger eye is consistently shorter. In a sample of 101 units, cells driven equally by the two eyes had nearly identical latencies through each eye (mean interocular difference: 2.2 msec), while units in OD groups 3&5 and 2&6 showed mean interocular latency differences of 7.1 and 13.1 msec respectively. Since simultaneous stimulation of the two eyes would result in asynchronous input to the cortical cell, units with interocular latency differences should respond best to stimuli which fell on the two receptive fields at slightly different times. To determine the sensitivity of units to the timing of input from the two eyes, we presented flashed stimuli to each eye with variable interocular delays. By varying space (position of stimuli on the two receptive fields) and time (interocular delay) simultaneously, it was seen that both factors influence the binocular responses of single cells.

All but a few units showed spatial interactions and, as described by other investigators, these responses showed summation, facilitation and/or inhibition. Sensitivity to interocular delay characterized many of the units encountered and those with interocular latency differences generally responded best with delays that represented simultaneity of input to the cortical cell. In some cells, inputs from the two eyes resulted in strong facilitation or inhibition only when they occurred within $\pm$ 10 msec of interocular synchrony. In other cells the range of temporal selectivity was broader, with units showing interactions over ranges of $\pm$ 30-50 msec. Units that were highly sensitive to the timing of input from the two eyes could discriminate 2 msec of interocular delay.

The interocular latency difference in cells driven unequally by the two eyes is similiar to that which can be produced psychophysically in humans by placing a dimming filter over one eye. In this case, one observes the Pulfrich phenomenon. It is proposed that a local Pulfrich-like phenomenon occurs in cat cortical cells activated binocularly with unequal latency. The units' sensitivity to interocular delay could provide the substrate for a time-based depth perception mechanism.

1781 A COMPUTER TECHNIQUE FOR MAPPING AND QUANTIFYING THE RETINAL PROJECTION ONTO THE SUPERIOR COLLICULUS. J.D. Gerard*, J.G. Pollack, and T.L. Hickey. School of Optometry/The Medical Center, University of Alabama in Birmingham, Birmingham, AL 35294

Autoradiographs of monkey superior colliculus show that axons arising from retinal ganglion cells end in discrete patches in the superficial layers. In an attempt to reconstruct, three-dimensionally, the pattern of retino-collicular input in the monkey, we have developed a computer technique which allows the position of each of the two-dimensional patches to be localized relative to its position in a given cross-section of the colliculus. When such information is obtained for serial sections it is possible to reconstruct, and quantify, the overall pattern of retino-collicular input.

Camera lucida drawings are made from either frontal or parasagital serial sections of paraffin embedded monkey superior colliculus. On each drawing the areas of retino-collicular input, as determined by the distribution of silver grains, are outlined as well as the overall outline of the colliculus and adjacent brainstem structures. Similar drawings are made for each of the 14μ thick serial sections throughout the rostral-caudal or medial-lateral extent of the colliculus. Once all camera lucida drawings are completed, the drawings are re-traced using a Graph-Pen system. This system makes it possible to digitize the position of each patch of retinal input relative to the surface of the colliculus. It is then possible to straighten the surface of the colliculus, keeping all underlying patches of retinal input in their relative positions but allowing them to be represented along a flat surface. By re-tracing each of the serial sections with the Graph-Pen, storing the digitized version of each section on computer discs and plotting out the flattened drawings sequentially, it is possible to look at the three-dimensional pattern of retinal input. Besides quantifying the overall extent of retinal input, this technique also allows us to look at the pattern of retinal input at different depths in the colliculus. Although we have only used this technique for looking at the three-dimensional pattern of retinal input to the colliculus, the technique is equally applicable to other structures. By using trans-synaptic autoradiography, it should be possible to map the three-dimensional pattern of cortical ocular dominance columns. Since this procedure could be carried out in both normal and visually deprived animals, it should also be possible to quantify any differences in geniculo-cortical input.

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1782 PROJECTIONS TO THE DIENCEPHALON AND MIDBRAIN FROM THE STRIATE CORTEX OF THE RABBIT: AN AUTORADIOGRAPHIC STUDY. R.A. Giolli, L.C. Towns, A.N. Karamanlidis*, T.T. Takahashi* and D.D. Williams* Dept. Anat., Sch. Med., UCI, Irvine, CA 92717 and Dept. Anat., Sch. Osteopath., Kirksville, MO 63501.

The projections of the rabbit's striate cortex (visual cortical area 1) to diencephalic and midbrain nuclei has previously been studied in detail by Giolli and Guthrie (J. Comp. Neurol., 142: 351-376, 1971) using the method of fiber degeneration. Because of the advantages that the autoradiographic method (ARG) has been reported to show over the fiber degeneration method, these projections of the rabbit's striate cortex have been reinvestigated using the ARG method.

An injection of ^{3}H leucine was made within sectors of the striate cortex in each of eighteen adult albino rabbits, and the brains were processed according to the method described by Cowan et al. (Brain Res., 37: 21-51, 1972). The results have confirmed the findings utilizing the fiber degeneration method by showing that the thalamic reticular nucleus, the ventral and dorsal lateral geniculate nuclei, the pulvinar (as defined by Rose, J. Comp. Neurol., 77: 469-523, 1942), the anterior and posterior pretectal nuclei and the superior colliculus receive inputs from the striate cortex. However, in disagreement with the observations using the method of fiber degeneration, the present study using the ARG method has revealed the following regarding the projections of representative sectors of the striate cortex: (i) both the ventrolateral nucleus and posterior thalamic nucleus (as defined by Rose (1942) are consistently seen to receive a cortical input and (ii) the nucleus of the optic tract, reported to be innervated by axons from the striate cortex in fiber degeneration studies (Giolli and Guthrie, 1971), is consistently found to lack a cortical input. (Supported by NIH grant 5RO1-EY00607).

1783 FUNCTIONAL PROPERTIES OF POSTERIOR PARIETAL CORTEX OF THE MONKEY. II. BEHAVIORAL ENHANCEMENT OF VISUAL RESPONSES. Michael E. Goldberg, David Lee Robinson, and Gregory B. Stanton. Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014

Neurons in areas 5 and 7 of the rhesus monkey have been described to discharge in association with eye and hand movements. We have shown that such neurons have visual and/or somatosensory responses that can be demonstrated in the absence of any active response on the part of the monkey toward the stimulus used to excite the neuron. When the animal used the stimulus as the target for an eye or hand movement, the discharge of some neurons in posterior parietal cortex can be enhanced.

Three rhesus monkeys were trained to fixate a spot of light, and then to make one of three responses: continue to fixate the spot and ignore any other visual stimuli which might appear on the screen; reach out and touch a panel should it be illuminated; make a saccadic eye movement to a small peripheral target. In this paradigm the same visual stimulus could take on three different meanings to the monkey: a target for a hand movement, a target for an eye movement, or an irrelevant, negligible stimulus. Extracellular single-unit recordings were done while the animals were performing their tasks. All behavioral control and on-line data analysis was done using a PDP 11-10 computer.

The visual responses of nearly half of the visually responsive neurons in areas 5 and 7 were brisker and more regular when the animal was going to make a saccadic eye movement to fixate or reach out to touch the target. This enhancement response was not entirely dependent upon the presence of the movement, because if in a series of trials the monkey made an occasional erroneous response, the enhancement was still present. The response, however, was absolutely dependent upon the presence of the stimulus. If the monkey made the proper movement under conditions when the proper stimulus was absent, the neuron did not discharge in relation to the movement.

The enhancement is spatially specific. When the animal sees two stimuli, one in the receptive field of the neuron and the other outside of the receptive field, the neuron has an enhanced discharge only when the animal makes a saccadic eye movement to fixate the stimulus in the receptive field. There is no enhancement when the animal makes saccades to a stimulus outside of the receptive field.

These data indicate that posterior parietal cortex acts as an association area for sensory and behavioral data, which processes may provide a physiological basis for the psychological process of attention. These data do not support the hypothesis that posterior parietal neurons command movement except to delineate an environment in which movement may be appropriate.

1784 INTERACTIONS AMONG RODS IN THE ISOLATED RETINA OF BUFO MARINUS. Edwin R. Griff* and Lawrence H. Pinto* (SPON: Jay Mittenthal). Dept. Biol. Sci., Purdue Univ., W. Lafayette, In 47907.

We searched for interactions among rods in the isolated retina of Bufo marinus by comparing the responses evoked by stimuli of different geometry. For each stimulus geometry, the luminous flux passing axially through the rod was measured. Circular stimuli of diameters 30 μm (small stimulus) and 500 μm (large stimulus) were adjusted such that equal flux passed through an impaled rod. Responses were recorded from rod outer segments. The peak response evoked by the large stimulus was always larger than the peak response evoked by the small stimulus. In addition, the sensitivity of a rod was measured to both small and large stimuli. After adjusting for the measured difference in illuminances of the large and small stimuli, the sensitivity of a rod to the large stimulus was 1.06 ± 0.27 (S.E.M.) log units greater than the sensitivity to the small stimulus. The larger response and greater sensitivity of the rods to the large stimulus must be mediated by the additional cells stimulated by the large stimulus. Thus, interactions among rods exist in the isolated retina.

Comparison of the time course of responses evoked by large and small stimuli of high illuminance allowed partial analysis of the mechanism mediating the interactions among rods. When stimuli were equated for equal flux through the rod, the response to the large stimulus reached a maximum faster than the response to the small stimulus. For the first 200 msec, the response to the large stimulus was more hyperpolarized than the response to the small stimulus. At about 200 msec, the response amplitudes were equal and, for the next 200 msec, the response to the large stimulus was less hyperpolarized than the response to the small stimulus. Interactions mediated solely by passive electrotonic spread cannot account for these response characteristics.

The contribution to the response made by rods at increasing distances from an impaled rod was analyzed by examining the responses of the impaled rod when a slit-shaped stimulus was presented at each of several displacements across the retina. A plot was made of the peak response amplitude vs. the displacement of the stimulus from the impaled rod. Data points fitted an exponential; for 10 cells, the mean space constant was 34.5 ± 6.9 (S.E.M.) μm.

Thus, in the isolated retina, a preparation used to study the mechanism of transduction, the response recorded from a single rod is not solely the result of quanta which stimulate that rod.

1785 ATTEMPTS TO REVERSE THE EFFECTS OF MONOCULAR DEPRIVATION IN THE ADULT CAT'S CORTEX W.A. Harris* and M.P. Stryker. Dept. Neurobiology, Harvard Medical School, Boston, Massachusetts 02115.

Monocular deprivation early in life causes the deprived eye to lose permanently its influence on most cells in the visual cortex (Wiesel and Hubel, J. Neurophysiol. 26:1003, 1963). Recent reports have suggested that this effect can be reversed either by enucleating the "normal" eye (Kratz et al., J. Neurophysiol. 39: 501, 1976) or by pharmacological means (Duffy et al., Nature 260: 256, 1976). The results are consistent with a model in which the spontaneous activity of the normal eye somehow inhibits responses from the deprived eye.

Retinal ganglion cell activity of the normal eye was silenced reversibly by raising intra-ocular pressure while single unit recordings were made in the lateral geniculate nucleus and the corresponding part of the visual cortex. The result was a reduction in spontaneous activity in the geniculate layers supplied by the blocked eye. Meanwhile, nearly all cortical cells studied remained uninfluenced by the deprived eye; most did, however, show reduced spontaneous activity against which it occasionally became possible to discern responses that had previously been masked by the background activity in both the unit studied and in smaller simultaneously recorded units. Enucleation of the normal eye produced similar changes to pressure blockade, although in the cortex changes in background activity of individual cells could not be assessed. Like those of Kratz et al. our cortical recordings with enucleation showed about one-third of the active cells to be influenced by the deprived eye. But the reduction in spontaneous activity seen with the pressure blockade leads us to interpret these results differently.

To examine further whether silencing the ganglion cells of the normal eye increases cortical responses to stimulation of the deprived eye (as suggested by Kratz et al.), an experiment was designed using Sokoloff's histochemical method for determining cerebral glucose uptake. A restricted lesion was placed in the retina of the normal eye by laser photocoagulation. This greatly reduced firing in the corresponding parts of the geniculate and cortex. We injected ^{14}C-2-deoxyglucose while visually stimulating the deprived eye. Autoradiograms showed somewhat reduced labelling density over the cortical region corresponding to the retinal lesion, probably reflecting a reduced activity in this region.

These experiments suggest that the ocular dominance distribution in monocularly deprived cats results not from tonic suppression by the normal eye, but rather from changes in the balance of excitation from the two eyes.

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1786 RESPONSES OF FISH RETINAL GANGLION CELLS TO MOVING CONTOURS. Thomas F. Hay* and Bruce H. Pomeranz. Dept. of Zoology, University of Toronto, Toronto, Ontario, Canada, M5S 1A1.

Fishes are used as subjects in many investigations of vision but there are relatively few reports on the tuning characteristics of single retinal ganglion cells. Metal-filled electrodes were used to record from ganglion cell terminations in the optic tectum of three species of fishes, goldfish, yellow perch and pumpkinseed sunfish. The electrode was placed so that receptive fields were located laterally and below the eye axis. At locations separated by at least 40 microns depth the activity of the largest single unit was isolated by a window discriminator and its response properties tested.

Of the 141 cells, 139 responded to dark, moving contours although 20 of these responded best to large objects or to darkening the field (dimming detectors). 86% evidenced clear velocity tuning, 64% showed object size preferences and 30% were directionally selective. Perch and sunfish had a smaller proportion of directionally selective cells than did goldfish, but more of their cells responded best to temporal-ward movement. Movement gated responses to stationary objects were rare; most cells responding to stationary objects were classified as dimming detectors. Depth within a penetration was not related to cell response properties in any clear or general manner. (Supported by NIH 1-F-32-N505400.)

1787 VARIABILITY IN PATTERNS OF LAMINATION IN THE HUMAN LATERAL GENICULATE NUCLEUS. T.L. Hickey. School of Optometry/The Medical Center, University of Alabama in Birmingham, Birmingham, AL 35294.

The patterns of cellular lamination have been studied in the dorsal lateral geniculate nuclei (LGN) of 53 human brains ranging in age from newborn to 40 years. Two fundamental patterns of lamination can be seen in frontal sections through the human LGN. The first pattern is characterized by 2 magnocellular layers and 4 parvocellular layers throughout much of the binocular segment of the nucleus. The second pattern, which is seen in approximately 25% of the LGN's studied, is characterized by a further splitting of one of the parvocellular laminae, resulting in 6 parvocellular layers (or leaflets) over a part of the binocular segment of the nucleus. Both patterns of lamination can be seen in neonates and adults, indicating that the differences are not a result of postnatal development.

Although the pattern of lamination varies within a single LGN as one moves from the rostral to caudal pole of the nucleus, the changes in the laminar patterns are usually quite predictable. In a few brains, however, this regular pattern of lamination is interrupted in the rostral third of the nucleus. In these brains the ipsilaterally innervated magnocellular layer 2 and parvocellular layer 3 can be seen ventral to the contralaterally innervated laminae 1 and 4. As one moves more caudally, the normal pattern of lamination appears with the exception that a few clusters of large layer 1 cells remain isolated between the parvocellular layers 3 and 4. Such isolated groups of large cells usually occur near the medial border of the nucleus and, by tracing through serial sections, can always be seen to merge with layer 1. This abnormal pattern of lamination has been seen in both frontally and parasagitally cut sections.

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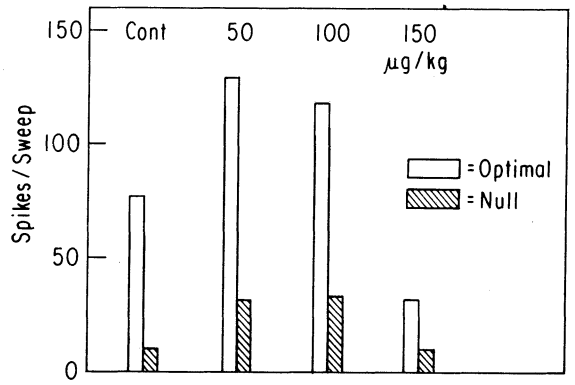
1788 EFFECTS OF LSD ON NEURONS IN VISUAL CORTEX. M. Hilmy*, B. Connors*, P. Fox and G. Somjen. Dept. Physiol. Pharmacol., Duke University Medical Center, Durham, NC 27710.

The impulse discharge of neurons was recorded in the visual receiving area of cats anesthetized with N_2O before and after the administration of lysergic acid diethylamide (LSD) (25 to 150 µg/kg wt cumulative, i.v. in two or three divided doses over several hours). Impulse histograms were time-locked to stimulus movement. Stimuli were luminous slits of various orientation swept back and forth over the receptive field. Of seven direction-selective cells (in 7 cats), the responses of four were depressed, of two enhanced at low but depressed at high dose (see figure) and of one unchanged. In cases of enhancement the response to non-optimal stimuli were more affected, reducing optimal/null response ratios, and unstimulated "background" was also increased. Overall effect of the drug may be a diminished signal-to-noise ratio, possibly the basis of distortions in visual sensory function. This may be a direct effect on the visual pathway, or a modification of input from non-visual subcortical sources. Our observations agree with those reported by Rose and Horn (Exptl. Brain Res. 27:71, 1977). (Supported by DA 01458)



Average responses of a single cell during a total of 5 hours.

1789 EFFECTS OF EARLY EXPERIENCE ON SPONTANEOUS ACTIVITY, RESPONSE VARIABILITY AND VISUAL RESPONSIVENESS OF NEURONS IN THE STRIATE CORTEX OF THE CAT. H.V.B. Hirsch & A.G. Leventhal. Center for Neurobiology, SUNYA, Albany, New York 12222.

The spontaneous activity, response variability and peak response to visual stimulation of neurons in the striate cortex of normal cats and of cats deprived of visual experience by dark-rearing (7-12 months from birth) were studied quantitatively.

Cortical cells in deprived animals displayed lower mean spontaneous discharge rates, greater response variability and lower peak responses than did neurons in normal cats. Significant numbers of neurons that were not responsive to visual stimulation were observed in deprived but not in normal cats. Thus, the visual responsiveness of cortical neurons is effected severely by visual deprivation.

In dark-reared cats virtually all orientation sensitive cortical cells have small response fields and respond only to stimuli moving slowly(low cutoff velocity); neurons with larger response fields and/or higher cutoff velocities do not display orientation sensitivity in these cats (Leventhal, A.G. & Hirsch, H.V.B., Proc. Nat.Acad.Sci. 74, 1272-1276, 1977). Two lines of evidence suggest that for some cells the loss of orientation selectivity is not simply the result of a drop in visual responsiveness. In dark-reared cats: (1) non-selective cells respond more strongly to visual stimulation than do orientation selective cells and, (2) non-selective cells respond strongly to stimuli of any orientation. Finally, while there are orientation selective neurons in the visual cortex of normal cats that respond to all stimulus orientations, their response to non-optimal stimuli is considerably weaker than: (1) their response to optimally oriented stimuli and, (2) the response of many non-selective cells in dark-reared cats to any stimulus orientation.

These results suggest that many non-selective neurons in dark-reared cats lack inhibitory inputs which, in normal animals, give rise to orientation sensitivity. A decrease in the efficacy of excitatory connections alone cannot explain why neurons with large response fields and/or high cutoff velocities in deprived cats respond well to all stimulus orientations while similar cells in normal animals do not. It is suggested that inhibitory connections in the mammalian visual system are especially dependent upon visual experience for the development or maintenance of normal function. (Support provided by USPHS Resaerch Grant R01 EY-01268 and Alfred P.Sloan Foundation Fellowship BR 1677).

1790 VISUAL RESPONSES OF NEURONS IN THE NUCLEUS OF THE OPTIC TRACT OF VISUALLY DEPRIVED CATS. K.-P. Hoffmann, K. Behrend and A. Schoppmann. Dept. Neurobiol. University Ulm (MNH), D-7900 Ulm, West Germany.

A specific class of neurons in the nucleus of the optic tract (NOT) in the pretectum of cats can be identified by the following criteria: (1) large pattern rich in contour are more effective visual stimuli than single spots or lines. (2) All cells are excited by movements from the periphery to the center of the visual field and inhibited by movements in the opposite direction. (3) Effective stimulus velocities in the preferred direction were within the range of $\leq 0,1 - < 50^{o}/s$. (4) Latencies to electrical stimulation of the optic chiasma lay between 4 - 6 ms. (5) All units sharing the above listed properties could be antidromically activated from the inferior olive. 60 % of the neurons recorded in the NOT of normal cats were binocularly driven. The remainder were activated from the contralateral eye only.

In adult cats of which one eye was kept closed from birth the properties of NOT neurons listed above remained largely unaltered except the neurons did not follow up to such high velocities and the inhibition by movements in a direction opposite to the preferred one was less pronounced in neurons driven from the deprived eye. There was however a complete loss of binocular influence onto the NOT, neurons in i.e. the NOT contralateral to the deprived eye were exclusively driven from the deprived eye and in the NOT ipsilateral to the deprived eye by the non-deprived eye.

Also after a lesion of the visual cortex direction selectivity and velocity tuning of neurons recorded in the NOT ipsilateral to the lesion remained largely normal. Again there was a complete loss of the influence from the ipsilateral eye.

In a normal cat an optokinetic nystagmus (OKN) can be elicited in the two horizontal directions even if one eye is stimulated alone. In the NOT on either side of the brain all cells prefer only movements from temporal to nasal in the contralateral hemifield. Each eye can however activate neurons in the two NOT preferring opposite directions of movement because of the binocularity of NOT neurons. If binocularity is lost due to deprivation or decortication each eye only activates the contralateral NOT. As a consequence OKN is only elicited by patterns moving in temporonasal direction if the deprived eye is stimulated alone.

1791 FOURIER ANALYSIS APPLIED TO SPATIAL PROPERTIES OF NEURONS IN THE CAT'S VISUAL CORTEX.
R.A. Holub, Robert Michael Jones* and Mary Morton Gibson. Depts. Neurophysiol. & Ophthalmol., Univ. of Wisconsin, Madison 53706.

We employed a Maxwellian view optical system to study the spatial properties of cells in the visual cortex of lightly anesthetized, paralyzed cats. Suprathreshold spatial frequency tuning curves measured with drifting sinusoidal gratings resembled spatially selective channels used to explain the results of various human psychophysical experiments. With rare exceptions, the responses of cells increased linearly with contrast up to saturating levels. The discharge pattern preserved the sinusoidal waveform of the input regardless of spatial frequency or drift-rate in many cells. For cells showing these two linear properties, Fourier analysis was used to compute the spatial frequency tuning curve expected from the responses to drifting edges and slits. The computed curves were compared to data recorded with sinusoidal gratings drifted at the same rate and of less-than-saturating contrasts.

For cells whose discharge pattern preserved the input waveform (these tended strongly to have 'simple' properties), the optimal spatial frequency was accurately predicted from either edge or slit responses. The curve computed from the PST histogram to the edge usually showed good agreement with the measured data at spatial frequencies below the optimum but was usually above the data at higher frequencies. Curves computed from slit responses had much more low frequency content than was measured. This discrepancy was eliminated and the computed curve brought into agreement with measured points if likely inhibitory zones of the receptive field were represented by negative discharge rates. The nature of these inhibitory zones was estimated by differentiating the PST's to edges, since the response to a narrow slit is the derivative of the response to an edge in a linear system. As might be expected from current thought about receptive field organization, similar attempts to simulate inhibitory regions in PST's to edges produced much less dramatic effects on the computed spatial frequency tuning curves.

We have found that Fourier analysis can be applied to the prediction of the effective sine-frequency-spectrum using responses to edges or narrow slits of light for cells whose output faithfully reflects a sine input. Thus, one can compute approximate responses to a wide variety of stimulus patterns from knowledge of the spatial frequency tuning curve.

This work was supported by NIH grants 4F01MH48,872 (RAH), NS06225 (Neuro) and EY00308 (U. Tulumay-Keesey).

1792 MICROELECTRODE ANALYSIS OF THE ORIENTATION COLUMN SYSTEM IN THE STRIATE CORTEX OF THE TREE SHREW (Tupaia glis). A.L. Humphrey, T.T. Norton and J.E. Albano, Depts. of Psychology and Physiology, Duke University, Durham, NC 27706.

An anatomical picture of the orientation column system in the tree shrew striate cortex has been obtained with deoxyglucose autoradiography (see abstract by Skeen et al., these meetings). In the present study we compare that picture to one obtained from microelectrode penetrations through striate cortex in paralyzed, N_2O anesthetized tree shrews.

We found that about half of the cells encountered within layer IV responded well to all stimulus orientations. This is consistent with the continuous horizontal strip of deoxyglucose uptake found throughout layer IV in animals stimulated with vertically oriented stripes. In contrast, most neurons above and below layer IV were selective to stimulus orientation. About half of these responded to stimuli deviating up to $\pm 20^{o}$ from the optimal orientation. On vertical penetrations we found that cells of similar optimal orientation were arranged radially in the cortex; from penetrations parallel to the cortical surface, we found orderly, progressive changes in optimal stimulus orientation. The slopes of the orientation change across cortex, together with the orientation tuning properties of the neurons, indicate that the width of the radial zones of increased deoxyglucose uptake correspond to the distribution of neurons expected to discharge to vertical stripes.

The slope of the orientation change with electrode movement parallel to the cortical surface varied considerably with the direction of that movement, suggesting an asymmetry in the representation of the orientation system. When we compared penetrations moving in various directions across cortex to the topographic maps obtained with the deoxyglucose method, we found that penetrations with high slopes ($250\text{-}600^{o}$/mm) ran generally perpendicular to the mapped iso-orientation lines, while those with low slopes (10^{o}/mm) ran generally parallel to the lines.

We conclude that the electrophysiological and the deoxyglucose techniques provide compatible and complementary pictures of the system for optimal stimulus orientation in Tupaia. The deoxyglucose method yields a better picture of the system's global organization, while the electrophysiological method yields a better picture of its microstructure.
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1793 A METHOD FOR SECURING ENHANCED ANTEROGRADE TRANSPORT OF HORSERADISH PEROXIDASE (HRP), USING THE RAT RETINOHYPOTHALAMIC PATHWAY. Stephen K. Itaya*, Terence H. Williams, and Edgar L. Engel. (SPON: J. Y. Jew). Dept. Anat., Univ. Iowa, Iowa City, IA 52242, and Dept. Anat., UTCHS, Memphis, TN 38163.

The first objective for the experiments reported below was to develop a reliable method for securing anterograde transport of HRP, and consistent results have hitherto eluded investigators. The second purpose was to use this method as a tracer to study the retino-suprachiasmatic pathway in detail. On the basis of the observation (Ryser and Hancock, Science 150:501, 1965) that poly-L-ornithine increases micropinocytosis in cultured cells, it was hypothesized that this agent would enhance uptake and subsequent anterograde transport of HRP by retinal ganglion cells.

Optimal results were obtained by 50 µl intraocular injections of 15 mg HRP (type VI, Sigma Chem. Co.) in 0.1 M phosphate buffer with one to ten µg/ml poly-L-ornithine (Sigma Chem. Co.), injected over a period of 30 min., provision being made for release of intraocular pressure. After 48 hrs., animals were perfused with 2% glutaraldehyde, 2% paraformaldehyde and 2% sucrose in 0.1 M phosphate buffer. Brains and optic nerves were immersed overnight in fixative, followed by a 24 hr. wash in buffer with 5% sucrose. Optic nerve and hypothalamus were isolated and 50 µm serial sections were cut with a Vibratome (Oxford Laboratories). Sections were incubated in 0.05% diaminobenzidine with 3% of 0.3% hydrogen peroxide in phosphate buffer for 30 min. on ice. Following 30 min. postfixation in 1% osmium tetroxide, the suprachiasmatic nucleus (SChN) was isolated from each section of hypothalamus and prepared for electron microscopy. Controls included (1) incubation without hydrogen peroxide and (2) injection of vehicle only.

Good results have been obtained in six of seven animals injected in the prescribed manner. With the light microscope, reaction product was evident in numerous optic nerve axons and was scattered bilaterally in the SChN, with most on the contralateral side. The electron dense, membrane bound reaction product was found in pre- and postsynaptic neural processes, at both symmetric and asymmetric terminals of the SChN. Controls were devoid of intraaxonal HRP.

Retinal ganglion cell synapses can be identified in the SChN by the modified technique for anterograde HRP transport which may have widespread application for tracing CNS pathways.

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1794 RESPONSES OF CELLS IN THE INFERIOR TEMPORAL CORTEX OF MONKEYS DURING VISUAL DISCRIMINATION REVERSAL. Charlene Drew Jarvis and Mortimer Mishkin, Laboratory of Neuropsychology, NIMH, Bethesda, Md. 20014

Cells in the inferior temporal cortex of awake monkeys can be driven selectively by stationary visual patterns and colors that the monkeys are required to discriminate (Jarvis and Mishkin, Neurosci. Abstr. 1: 61, 1975). In the present study we asked whether the responses of these cells could be modified by changing the motivational significance of the stimuli. To explore this possibility, two rhesus monkeys were trained on a task in which a white ring (the positive stimulus) was paired with each of 24 negative stimuli in separate blocks of trials. The two stimuli were presented successively in random order on the left of two response keys. A correct response to the positive stimulus (left-key press) resulted in juice reward, while a correct response to the negative stimulus (right-key press) led only to advance of the random stimulus generator. If either stimulus was found to be effective in driving a cell, the reward values of the two stimuli, and the response requirements associated with them, were reversed. If the reversal resulted in a change in the firing characteristics of the cell, one or more additional reversals of that pair were given before other stimulus combinations were presented. Ninety-eight cells were studied using this paradigm. Eighty of these cells showed no apparent changes in their firing characteristics during reward reversal. Of the 18 cells that did show changes, only a few seemed to be sensitive to shifts in stimulus-reward association per se. The others were excluded from this category either because they showed changes in spontaneous discharges rather than in stimulus-locked activity, or because their changes in stimulus-locked activity appeared to reflect differences in the duration of fixation of the same stimulus in the two reward conditions. The data suggest that inferior temporal neurons are more concerned with the physical properties of stimuli than with their motivational significance. By implication, the visual impairment after inferior temporal lesions is more likely to reflect a disorder in stimulus processing than in stimulus-reward association.

1795 VISUAL RESPONSES DURING SACCADIC EYE MOVEMENT: VISUAL MASKING IN STRIATE CORTEX. Stuart J. Judge and Robert H. Wurtz, Laboratory of Neurobiology, National Institute of Mental Health, Bethesda, Maryland 20014.

Each saccadic movement of the eyes smears across our retinae the image of the scene before us, a fact of which we are normally unaware. If the scene is illuminated only during eye movement, however, the smearing is clearly perceived. Thus masking by stimuli present on the retinae before and after each saccade must account for our lack of perception of the saccadically smeared image (Wurtz and Campbell, 1977). We have looked for a physiological correlate of this masking effect in the striate cortex of the awake monkey, Macaca mulatta, trained to perform a visual fixation task. We recorded from striate cortex representing the lower visual field between 5 and 15 degrees from the fovea. Approximately one third of the neurons isolated responded to stimuli moving at saccadic velocities. These neurons responded similarly whether the retinal stimulation was produced by rapid movement of a stimulus across the stationary receptive field or by a saccadic movement of the eye which swept the receptive field across a stationary stimulus. The responses of such neurons to a stimulus swept across their receptive field by a twenty degree saccade (a sweep stimulus) could be attenuated or extinguished by stimulation of the receptive field before and/or after the saccade. (The stimuli were 10 cd/m^2 spots of light on a background of 1 cd/m^2.) Stimuli present before the saccade completely abolished the response of about 90% of neurons to the sweep stimulus, whereas stimuli falling on the receptive field after the saccade abolished the response in only about 30% of the neurons. When the monkey made no eye movement and a stimulus was swept across the receptive field before the onset or after the offset of a stationary receptive field stimulus, there was a similar attenuation of the response to the sweep stimulus, which was more sustained when the stationary stimulus preceded the sweep stimulus. Precise alignment of the stationary stimulus on the center of the receptive field of the neurons was not necessary to produce such response attenuation effects: elimination of the response to the sweep stimulus was observed using stationary stimuli which fell on the surround of the receptive field. This physiological effect may underlie the perceptual masking of the smeared retinal image produced by saccadic eye movement.

1796 EFFECTS OF MONOCULAR DEPRIVATION ON THE GROWTH OF LATERAL GENICULATE CELLS IN THE CAT. R.E. Kalil, Dept. of Anatomy, Univ. Wisc., Madison, WI. 53706

Although there is abundant evidence concerning the influence of monocular deprivation on geniculate cell size in the cat, little is known concerning the development of these effects. To study this question, the lids of one eye in 21 kittens were sutured shut between 7 and 10 days after birth. Following survival periods that ranged from 2 to 32 weeks (3,4,8, 12 and 16 weeks intervening), the cats were perfused with 10% formalin. For comparison, 2 kittens were deprived for 16 weeks by suturing shut the nictitating membrane of one eye. The brains were embedded in celloidin, sectioned in the frontal plane at 20µ, and stained with cresyl violet. Near the rostrocaudal midpoint of each lateral geniculate, the areas of 100 cells from lamina A1, and from the binocular and monocular segments of lamina A were measured at 1000x. The brains of 3 cats were examined at each survival period. Not until four weeks after lid suture is it possible to detect a significant difference in size between deprived and normal geniculate cells. At this time, deprived cells are approximately 16% smaller than normal. After longer periods of deprivation, cell size differences first increase, reaching a maximum at 12 and 16 weeks (26% for binocular A cells) and then decline slightly at 32 weeks (20% for binocular A cells). Closing the nictitating membrane for 16 weeks leads to cell size changes equivalent to those produced by lid suture. Comparing the percent shrinkage of deprived binocular and monocular A cells at each survival period indicates that at 8,12,and 16 weeks binocular A cells are affected by monocular lid suture more severely than monocular A cells. The differential effect is slight, however, averaging only about 6%, and it is not found in the long term deprived animals that survived for 32 weeks. Cell growth curves show that the consequences of monocular lid suture are threefold. Firstly, cell growth in deprived laminae is halted during the 8 weeks immediately following lid closure. Secondly, between 8 and 16 weeks,deprived cells undergo a progressive atrophy and are smaller at 4 months after lid suture than at 2 months. Finally, between 16 and 32 weeks there occurs a partial recovery of size which is most pronounced in deprived binocular A cells. In summary, eyelid suture abruptly arrests geniculate cell growth and this immediate effect is followed, approximately 2 months later, by a period of atrophy from which limited long term recovery is possible. Monocular deprivation appears to affect binocular segment cells slightly more severely than monocular cells, but only for intermediate length periods. When deprivation is prolonged, differential binocular segment-monocular segment effects are abolished.

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1797 LAMINAR SEPARATION OF ON AND OFF COMPONENTS OF THE PROXIMAL NEGATIVE RESPONSE AND K^+-FLUX IN FROG RETINA
Chester J. Karwoski* and Luis M. Proenza, Vision Res. Lab., Dept. of Psychol., Univ. of Ga., Athens, 30602

Field potentials and changes in extracellular potassium concentration ($[K^+]_o$) were recorded from the retina of the frog (Rana pipiens) in response to a wide variety of photic stimuli. As previously reported by Oakley & Green (J. Neurophysiol., 39:1117, 1976), light stimulation induces a decrease in $[K^+]_o$ in the distal retina and an increase proximally. We further characterize the proximal K^+-increase by showing that it is maximal with small spots, is very sensitive to stimulus position, and exhibits ON as well as OFF components. Thus, the stimulus dependent behavior of the K^+-increase in frog is quite similar to that previously reported by us in mudpuppy (J. Neurophysiol., 40:244, 1977).

In frog, a species with a thick, well-developed inner plexiform layer (IPL), the maximum K^+-increase and proximal negative response (PNR) at light onset occur at about 33% retinal depth (within the IPL), whereas maximum K^+ and PNR responses at light offset are registered some 25 μm more distal, at 42% (which in frog is the boundary of the IPL and inner nuclear layer--INL). These results support the proposal of Holden (J. Physiol, 221:173, 1972) in the pigeon, and of Kolb, Famiglietti, & Nelson (Science, 194:193, 1976; & personal communication) in cat, that ON and OFF systems are represented in separate sublaminae of the IPL, with the OFF system being more distal.

In mudpuppy, a species with a thin, poorly developed IPL, maximum K^+-increase and PNR at both light onset and offset occur at about 20% retinal depth (which in mudpuppy is the boundary of the IPL & INL). If a differential depth distribution of the ON and OFF components of the PNR and K^+-increase exists in mudpuppy, it must be on the order of less than 10 μm.

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1798 A QUANTITATIVE STUDY OF ASCENDING AND DESCENDING INPUTS TO THE RABBIT LATERAL GENICULATE NUCLEUS AND SUPERIOR COLLICULUS.
Amy S. Kelly* and Patricia C. Fox (SPON: K. L. Chow). Dept. Neurol., Stanford U. Med. Ctr., Stanford, CA 94305.

We have used the method of retrograde transport of horseradish peroxidase to study the distributions of cortical and retinal cells which provide inputs to the rabbit lateral geniculate nucleus (LGN) and superior colliculus (SC). Peroxidase (.5 μl, 10% solution in 0.5 M NaCl) was pressure injected through a recording micropipette into retinotopically identified sites in the LGN or SC in different animals. Whole mounts of retinas were processed, and the distributions of labelled ganglion cell body sizes were compared. A diverse group of ganglion cells were found to project to SC, including both large and small cells. A more homogeneous group of small to medium sized cells were labelled with LGN injections. These results can be correlated with functional differences known to exist in the postsynaptic cell populations [Chow et al., Brain Res. 33, 337 (1971)], and with recent results from axonal transport work (Wagner, Kelly and Kelly, Neurosci. Absts., 1977) which show that different proteins are transported in optic nerve fibers going to LGN and SC in the rabbit. In striate cortex (VI), large pyramidal cells in the superficial one-half of layer V were labelled with SC injections. About 50% of the cells in the labelled region contained label. Smaller pyramidal cells in the superficial one-half of layer VI were labelled with LGN injections, and approximately 75% of the cells were labelled. The corticofugal projections in rabbit are thus more restricted within sublayers than in some other species. These data can also be related to receptive field properties of cells in layers V and VI.

1799 SUPRATHRESHOLD VISUAL TEMPORAL INTEGRATION IN HUMANS: A COMPARISON OF REACTION TIME AND TWO-PULSE DISCRIMINATION RESPONSES.
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The characteristics of temporal integration or summation (Bloch's law or stimulus intensity-time reciprocity) were measured and compared for two distinct responses--simple reaction time (RT) and two-pulse discrimination--in human observers. Temporal integration was measured for both responses by using a non-typical stimulus array consisting of two sequentially presented pulses of light of fixed luminance separated by a variable (in msec) interpulse interval of darkness, i.e., a two-pulse stimulus array. The independent variable was the duration of the two-pulse array measured from the onset of the first pulse to the offset of the second pulse. The light pulses transilluminated a white-appearing, foveally-fixated target that subtended a visual angle of 21'. The RTs were simple finger lifts to the double-pulse stimuli, measured from the onset of the first pulse of light. The two-pulse discrimination task consisted of a temporal forced-choice procedure in which the observers were required to discriminate various double-pulse stimuli of the same luminous energy from a brief (4-msec) equal-energy, fully-integrated, single pulse of light.

Results showed that all observers displayed several of the expected characteristics of temporal integration for both responses: complete integration (stimulus intensity-time reciprocity); partial integration; and a region of no integration, i.e., a region for the longer duration stimuli where changes in the stimulus did not affect the response. A partial integration index showed for the same stimuli more partial integration for discrimination than for RT. Stated differently, utilization time (the longest stimulus duration displaying partial integration) was briefer for RT than for two-pulse discrimination.

A review of other psychophysical and neurophysiological studies measuring both response latencies and response frequencies, or their equivalents, suggests that response latencies are most meaningfully related to the latency of the underlying neural response while response frequencies are related to the amplitude of the underlying neural response.

1800 AMACRINE CELL CONTRIBUTION IN THE CENTER RESPONSE OF CAT RETINAL GANGLION CELLS.
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Gamma-aminobutyric acid (GABA) can be localized to amacrine cells in the cat retina, suggesting that a subpopulation of amacrine cells utilize GABA as a transmitter. It has been previously reported that bicuculline, a GABA antagonist, has a substantial effect on center-surround organization in Y-type cat retinal ganglion cells. Surround dominated responses are greatly reduced or abolished while center dominated responses are somewhat less reduced. The net effect of the GABA antagonism is to shift center-surround balance in favor of the center. It was reported in 1975 at this meeting that the physical dimensions of the center, as estimated by area-threshold curves, are also reduced following bicuculline administration. Since the area-threshold curves on which this was based were determined by auditory thresholds of ganglion cell firing, and if correct as previously reported implicate amacrine cells in the organization of receptive field centers as well as surrounds, an objective determination of the same information was required.

Using a PDP-11 computer programmed to determine ganglion cell threshold from extracellularly recorded single units in the optic tract of anesthetized cats, the area-threshold experiments were repeated giving an objective determination of ganglion cell center size. Since the choice of a threshold criterion is arbitrary, threshold here was selected as the stimulus strength necessary to produce an average of three extra spikes in the response. Because the three extra spikes were measured in a 100 msec window, a threshold response would therefore be one of 30 spikes/sec, well within the linear stimulus-response range. The results, i.e., that the diameter of the receptive field center is reduced following GABA antagonism, confirmed those previously reported and support the conclusion that at least a portion of the center signal from Y-type receptive fields in cats reaches the ganglion cell through amacrines.

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1801 ANALYSIS OF MAINTAINED SPIKE DISCHARGES IN THE CAT VISUAL PATHWAY DURING DARKNESS AND STEADY LIGHT, Wlodzimierz M. Kozak, Dan Schweitzer-Tong*, Arthur C. Sanderson*, and Jakub Segen*. Biomed. Eng. Prog., Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213. (SPON.: J. R. Boston).

Spike trains in single Optic Tract (OT) Fibers and Lateral Geniculate (LGN) neurons were recorded extracellularly during darkness and six levels of steady diffuse illumination (5×10^{0} - 5×10^{-5} ftL) in Urethan-anesthetized cats. To insure that the data were reliable, each luminance level was repeated at least twice. Single units were categorized according to their respective field sizes and discharge properties. Monotonic relationship between mean rate and luminance level was seldom encountered, and instead, in the 10^{-4} - 10^{-3} ftL range, a maximum or minimum was often observed. For similar mean rates at high and low luminances significantly different temporal spike distributions were frequently found. For a given luminance, characteristic types of distributions were regularly and repeatably elicited. Simultaneous recordings from the input (unitary Excitatory Synaptic Potentials) and output (soma spikes) of single LGN neurons revealed several types of spike train transformation at these synapses.

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1802 EFFECTS OF DARK REARING ON PHYSIOLOGY AND ANATOMY OF THE CAT'S LATERAL GENICULATE NUCLEUS. K.E. Kratz, R. Kalil, and S.M. Sherman, Dept. Physiol., Univ. VA Med. Sch., Charlottesville, VA and Dept. Anat., Univ. Wisc. Med. Sch., Madison, WI.

Electrophysiological recordings were made from 156 single LGN neurons in 4 cats dark reared from birth to 4 months of age. Two of the cats were studied immediately upon removal from the dark with no visual experience, while the two remaining animals were given 13 or 28 months of normal light rearing before recording. Cells in laminae A and A_1 were sampled and were classified as X or Y on the basis of conduction velocity, receptive field linearity, etc. Using the same criteria with identical techniques in normal cats, we found that 50-60% of LGN neurons were Y-cells. Of the 156 LGN cells studied in the dark reared cats, 120 (77%) were normal X-cells, 30 (19%) were abnormal or unresponsive, but only 6 (4%) were normal Y-cells. This represents a significant reduction in the normal proportion of Y-cells ($p<0.001$ on a χ^2-test). No significant inter-animal differences were noted (e.g., the range of Y-cells for the 4 cats was 0-7%), and the dark reared-light reared cats exhibited roughly the same percent of Y-cells (6%) as did the cats with no visual experience (2% Y-cells; $p>0.2$ on a χ^2-test). As previously reported (Sherman et al., *J. Neurophysiol., 35:* 532, 1972), we conclude that visual deprivation affects Y-cells more than it does X-cells. However, dark rearing has a much more profound effect than does binocular lid suture (4% Y-cells vs. 29% Y-cells; $p<0.001$ on a χ^2-test), presumably because lid suture permits considerable, unpatterned light to strike the retina. Finally, histological measurements were made of cell area and packing density in 5 normal controls, 6 cats dark reared to 16 weeks and sacrificed, and 3 cats dark reared to 16 weeks and then light reared under normal conditions for 4 to 18 months. No histological differences among groups were found. It thus seems unlikely that the reduction in Y-cell percentages caused by dark rearing are due to simple changes in electrode sampling characteristics brought about by abnormal cell sizes or packing densities. We therefore conclude that dark rearing causes a marked reduction in the percent of Y-cells in the LGN. This effect is not reversed by subsequent periods of prolonged light rearing nor is it mirrored anatomically by any apparent changes in cell size.

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1803 SPATIO-TEMPORAL PROPERTIES OF S-POTENTIALS IN CATFISH RETINA. Howard I. Krausz. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Previous studies of horizontal cell potentials (S-potentials) have focused exclusively on either temporal properties or spatial properties. Temporal dynamics have been measured in various retinas using intensity modulated light spots. Spatial properties of S-potentials have been measured by recording the steady-state voltage at various distances from the center of a spot or slit of light with constant intensity.

The present work is an attempt to integrate both temporal and spatial properties into a simple yet comprehensive model able to account for two new findings:

1. As the diameter of a white-noise modulated light spot is increased, the high frequency gain also increases.
2. As the stimulating spot is moved away from the recording electrode, the overall gain first increases noticeably and then declines.

By treating the horizontal cell layer as a flat conducting disk (S-space) with feedback onto nearby receptors it is possible to obtain a closed-form solution for the spatio-temporal transfer function of the receptor→horizontal cell system. The model accounts for the findings above and makes other testable predictions.

1804 THE PATHWAY FROM THE CAT'S SUPERIOR COLLICULUS TO THE PARABIGEMINAL NUCLEUS: A STUDY OF ITS CELLS OF ORIGIN.
T. Langer*, H. Sherk, and A. Graybiel. Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

The cat's parabigeminal nucleus has been shown to receive a dense projection from the superficial layers of the superior colliculus, and in turn to project densely to the upper part of the superficial collicular layers on both sides. We have examined the population of neurons in the cat's superior colliculus that project to the parabigeminal nucleus. Our aims were to determine the laminar distribution of these neurons and to characterize their morphology.

In order to identify these cells, we have used the retrograde horseradish peroxidase (HRP) cell-labelling technique. HRP was injected electrophoretically into the parabigeminal nucleus of 4 cats (bilaterally in 1) after the nucleus was located by recording visual cell responses. Brains fixed by mixed aldehyde perfusion were prepared by modified Graham-Karnovsky (DAB and BDH) protocols.

Cells in all layers of the superior colliculus were labelled after HRP injections in the parabigeminal nucleus, but the majority (about 70%) were in the superficial gray layer. Labelled neurons were most numerous in the lower two-thirds of this layer, a zone receiving inputs from the ipsilateral retina and visual cortical areas. Fewer labelled neurons appeared above this, in the tier receiving dense inputs from the contralateral retina and the parabigeminal nuclei. About 30% of the labelled collicular cells lay deep to the superficial gray layer, for the most part in the stratum opticum and intermediate gray layers but also in the deep layers and central gray substance (ca 5%.) Throughout the colliculus, small to intermediate sized cells were labelled. A variety of neuronal types were labelled, but in the superficial layers many HRP-positive neurons had vertically aligned dendrites, while in the deeper layers stellate cells predominated.

The finding of labelled neurons in the deep as well as in superficial layers is notable because the projection from the parabigeminal nucleus to the superior colliculus terminates mainly, if not exclusively, in the superficial layers. These deep neurons were labelled after even the smallest injections, which were confined to the lateral half of the parabigeminal nucleus. It is possible that tectal fibers passing lateral to the nucleus were responsible for labelling of deep-layer collicular neurons; however, this cell-labelling could indicate instead the existence of an indirect pathway from the deep to superficial layers of the superior colliculus.

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1805 BILATERAL RETINO-WULST PROJECTIONS IN FALCON REVEALED BY TRANSNEURONAL TRANSPORT OF ^{3}H PROLINE. Stephen Lehmkuhle*, V. A. Casagrande, and Robert Fox. Departments of Anatomy and Psychology, Vanderbilt University, Nashville, Tennessee 37240.

Stereoscopic depth perception has been demonstrated by behavioral methods in the American Kestrel, a small falcon with bifoveate retinae (Fox et al., Neur. Sci. Abs. 2: 1075, 1976). We now report anatomical investigations of the Kestrel visual system using autoradiographic techniques. To examine retinal projections to first order mesencephalic and diencephalic structures we injected 0.5 Mc of ^{3}H proline into the vitreous of one eye and allowed the birds to survive for 24 hours. To measure transsynaptically transported labelled protein to the telencephalon, we injected intraocularly a total of 4.5 Mc ^{3}H proline and allowed the bird to survive for 14 days.

Following a short survival time, label was present only in regions contralateral to the injected eye; specific structures containing dense label were the OPT complex, the superficial layers of the tectum, the pretectal nuclei, the accessory optic nuclei, and the ventral lateral geniculate nucleus. Following a long survival time transsynaptic transport occurred depositing labelled protein in both the ipsilateral and contralateral areas of the Wulst. The contralateral label formed a dense, extended, continuous band within the posterior Wulst. The ipsilateral label was lighter and appeared to be divided into a medial and lateral segment. The medial segment was anterior to the lateral segment and slightly more ventral than the contralateral label. Transsynaptically transported label was also observed in the ipsilateral OPT complex, bilaterally in nucleus rotundus, ipsilaterally in the isthmic nuclei and in the mesencephalic reticular formation and pons. Above background label was also observed in all zones lying adjacent to heavily labelled retinal targets. Above background label was not observed within the ectostriatum.

These results, which demonstrate binocular innervation of the Wulst, are quite congruent with behavioral evidence for stereopsis. Moreover, the general organization of the falcon visual system is highly similar to that found in pigeon and owl by degeneration methods (e.g. Karten et al., JCN 150: 253, 1973). The overlapping and displaced portions of the contralateral and ipsilateral label correspond to the incomplete segregation of ocular input implied by electrophysiological recording from the Wulst of pigeon (Cuenod, The Neuroscience 3rd Study Program: Chap. 2, 1974). Finally, the results lend support to the general hypothesis that birds with binocular visual fields and perhaps all binocular vertebrates are equipped with pathways which permit extensive exchange of information from both eyes. (Supported by EY01778 and EY00931).

1806 POSTNATAL DEVELOPMENT OF OCULAR DOMINANCE COLUMNS IN LAYER IV OF THE CAT'S VISUAL CORTEX S. LeVay, M.P. Stryker, and C.J. Shatz. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

The segregation of geniculocortical fibers representing the two eyes was studied by transneuronal transport of radioactive proline injected into one eye, and by physiological recordings. As late as 15 days postnatally radioactive label formed a continuous band in layer IV on both sides of the brain, suggesting that afferents serving the two eyes are intermingled. At 22 days of age, periodic variations in grain density first appeared, but only on the side ipsilateral to the injected eye. This variation was more distinct at 33 days and achieved the adult appearance at 39 days. On the contralateral side such variation was not apparent until 39 days.

Interpretation of these findings was complicated by the possibility that significant amounts of radioactivity might spill over into the inappropriate laminae of the lateral geniculate nucleus and label the wrong set of cortical afferents. This was studied in autoradiograms of 1 μm Epon sections by measuring the ratio of labelling density over neuronal nuclei in laminae A and A1. We found that this spillover was always greater on the contralateral side, and greatest in the youngest animals. When spillover was taken into account quantitatively in the interpretation of grain counts made from the cortical autoradiograms, three findings emerged: 1) At 8 and 15 days, the continuous appearance of radioactive label on the ipsilateral side is not due to spillover but reflects a real uniformity in the distribution of ipsilateral-eye afferents. 2) The continuous pattern of label seen prior to 39 days on the contralateral side is uninterpretable owing to spillover. Other considerations, however, make it seem likely that the process of segregation proceeds synchronously for the two sets of afferents. 3) In the adult each eye's afferents occupy the centers of the appropriate ocular dominance columns almost exclusively, but they overlap at the borders.

The late onset of segregation (as compared with the monkey, Rakic, Nature 261:467, 1976; Hubel, Wiesel, and LeVay, Phil. Trans. Roy. Soc. B. 278:377, 1977) allowed us to examine whether transient functional connections are originally made uniformly along layer IV, in an unsegregated pattern. Tangential penetrations through layer IV in young kittens showed most of the cells to be nearly equally driven through the two eyes, in contrast to the adult (Stryker and Shatz, Neurosci. Abstr. 2:1645, 1976). This finding suggests that functional connections may be broken and reformed during development. It is striking that the onset of segregation coincides roughly with the beginning of the critical period (Hubel and Wiesel, J. Physiol. 206:419, 1970). Supported by NIH grants EY R01 1960, EY0082, EY05172

1807 LAMINAR DIFFERENCES IN RECEPTIVE FIELD PROPERTIES OF CELLS IN CAT VISUAL CORTEX. A.G. Leventhal and H.V.B. Hirsch. Center for Neurobiology, SUNYA, Albany, New York 12222.

Receptive field properties of neurons in the visual cortex of normal adult cats were analyzed quantitatively. Neurons were classified into one of two groups: (1) S-cells, which have discrete excitatory and inhibitory regions in their receptive fields and/or display "on" and/or "off" responses to flashing stimuli; (2) C-cells, which do not possess discrete excitatory and inhibitory regions in their receptive fields and give an "on-off" response or no response to flashing stimuli.

S-cells in a given lamina have smaller response fields, lower cutoff velocities, lower preferred velocities, and lower peak responses than do C-cells in the same lamina. In addition, S-cells, regardless of their laminar location, display lower relative degrees of binocularity and are more selective for stimulus orientation than are C-cells. Many of the receptive field properties of cortical cells vary with laminar location. Within the class of S-cells, receptive field size, cutoff velocity and preferred velocity increase with depth (layers V-VI) in the cortex. However, S-cells in all layers of the cortex display similar orientation sensitivities, mean spontaneous discharge rates, peak responses and degrees of binocularity. Laminar differences also exist within the class of C-cells. C-cells in layers V-VI display high mean spontaneous discharge rates, weak orientation preferences and high relative degrees of binocularity when compared to C-cells in layers II-III. The receptive field sizes, cutoff velocities and preferred velocities of C-cells increase with depth (layers V-VI) in the cortex.

The present results are compatible with the suggestion that most S-cells in the striate cortex receive LGNd X cell afferents and that most C-cells receive afferents from Y cells in the LGNd. The small relative receptive field sizes and low relative cutoff velocities of C-cells in layers II-III, as well as the large relative receptive field sizes and the high relative cutoff velocities of some S-cells in layers V-VI, are not reconciled easily with this hypothesis. The receptive field properties of cells in layers V-VI of the striate cortex suggest that LGNd Y cells project more heavily to the lower cortical layers than to the upper ones, thus preserving the distribution of Y cells found in the LGNd. (Support provided by USPHS Research Grant R01 EY-01268 and Alfred P. Sloan Foundation Fellowship BR 1677)

1808 EFFECTS OF VISUAL DEPRIVATION ON THE CAT'S GENICULO-CORTICAL PATHWAYS. C.S. Lin and S.M. Sherman, Dept. Physiol., Univ. VA Med. Sch., Charlottesville, VA.

Horseradish peroxidase (HRP) was injected focally into cortical area 17 or 18 of normal and monocularly deprived (MD) cats to assess the size and distribution of LGN cells projecting to these areas. After bilateral area 17 injections in one normal cat, 68-76% of the LGN cells (mostly smaller and medium size) were labelled; after bilateral area 18 injections in another normal cat, 24% of the cells (mostly large) were labelled. In 2 MD cats area 17 was injected with HRP in both hemispheres; all LGN layers had 63-74% of the cells labelled. Therefore, although labelled cells in the deprived laminae were somewhat smaller than those in non-deprived laminae, roughly equal numbers of labelled neurons were found in all laminae. In 3 other MD cats, HRP injections were limited to area 18, one in the hemisphere contralateral to the deprived eye, and two, ipsilateral to this eye; but results from both sides were indistinguishable and are considered together. Nondeprived laminae had 22-27% labelled neurons, but labelling in deprived laminae was very light and at most included 11-13% of the neurons which represents a significant reduction. However, the labelled cells in deprived laminae were only 13-14% smaller than those in nondeprived laminae. Two other MD cats were raised with a small retinal lesion in the open eye to produce an artificial monocular segment (AMS) for the deprived eye. This AMS and surrounding region of area 18 was injected in these cats. Labelling in the LGN was essentially limited in the deprived laminae to the AMS region and was found in the nondeprived laminae only outside the zone of transneuronal degeneration associated with the lesion. We conclude that in MD cats the deprived LGN connections to area 17 seem less disrupted than those to area 18 in terms of the percentage of labelled cells. Since X-cells project only to area 17 whereas Y-cells project both to areas 17 and 18 (Stone and Dreher, J. Neurophysiol., 36: 532, 1973), this suggests that the Y-cell projections are more affected by the deprivation than are those of X-cells (Sherman et al., J. Neurophysiol., 35: 532, 1972). Finally, since labelling was intense in the deprived AMS of LGN after injections of HRP into area 18, this supports the contention that development of these Y-pathways is dependent upon competitive interactions between pathways from each eye (Sherman et al., Brain Res., 100: 441, 1975).

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1809 CAT RETINAL GANGLION CELL SENSITIVITY AND RETINAL OXYGEN TENSION DURING HYPOXIA. R.A.Linsenmeier*, C. Enroth-Cugell, and T.K. Goldstick*. Biomedical Engineering Center, Northwestern University, Evanston, IL. 60201.

Previous work has shown that the massed optic nerve discharge is reduced during hypoxia, but the work reported here is the first attempt to study single ganglion cells during mild hypoxia.

Recordings were made from X and Y cells in the optic tract of anesthetized, paralyzed adult cats. The stimulus was a sinusoidal grating of relatively high spatial frequency, drifting across the receptive field so that the temporal modulation remained constant at 2 Hz. A computer collected PST histograms of the cell's discharge (which was sinusoidally modulated at the drift frequency), measured the peak-to-peak response amplitude, and adjusted the contrast of the grating to maintain a criterion response. At the spatial frequency chosen, the criterion response of 20 spikes/sec could be elicited by about 10% contrast before hypoxia. Repeated determinations showed that the contrast sensitivity (the reciprocal of the required contrast) was stable. Hypoxia was then induced by changing the breathing gas, and sensitivity was tracked during and after hypoxia. Simultaneously the oxygen tension in the vitreous humor 100 to 200 microns from the retina (Pr) was measured with a Clark type oxygen electrode. Intermittent measurements of arterial oxygen tension (Pa) were also made.

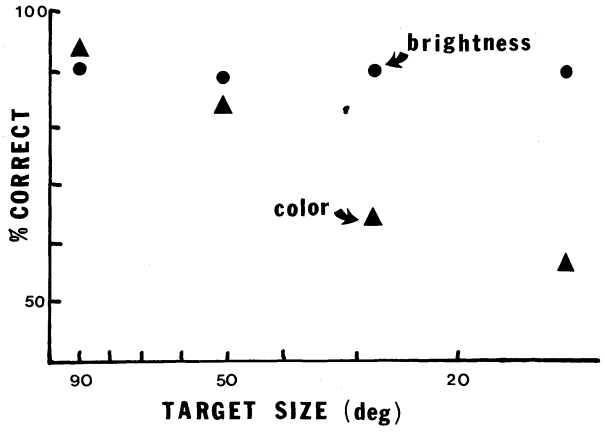
The time course of changes in sensitivity and Pr was similar, but not identical. When sensitivity changes occurred they usually began about 3 min. after the onset of hypoxia. Pr began to change in about 30 sec. and reached a hypoxic steady state in 2 to 3 min. When, following hypoxia, the animal was returned to air breathing, sensitivity and Pr recovered rapidly, but Pr overshot the initial value by as much as 100% before stabilizing.

Sensitivity did not always change. In fact the results indicate that ganglion cells are quite resistant to hypoxia. No change in sensitivity was observed provided that Pa was above about 35 mm Hg. Below this value of Pa sensitivity was reduced by varying amounts, but frequently the change was so great that the criterion response could not be elicited even with a stimulus contrast of 80%. The maintainance of normal sensitivity appears to depend on the efficiency of retinal oxygen autoregulation. The relation at steady state between Pa and Pr can be well described by a function consisting of two parts: 1) As Pa drops from 100 to 35 mm, Pr is well regulated, dropping from about 20 to about 14 mm Hg. 2) As Pa decreases further, Pr drops precipitously, reaching zero at a Pa of about 20. Thus the large sensitivity changes occur when the retinal circulation is unable to maintain retinal oxygen tension.

Supported by NIH 5-RO1-EY00206, 5-TO1-GM00874, and the Rowland Foundation.

1810 EFFECT OF TARGET SIZE ON COLOR DISCRIMINATION IN THE CAT. Michael S. Loop and Laura Bruce. Neural and Behav. Biol. Program and Dept. of Physiol. & Biophysics, Univ. of Ill., Urbana, Ill. 61801

The "problem" of cat color vision exists because both negative and positive findings have been reported and positive findings have generally suggested that the discrimination of color is, at best, very difficult for the cat. Recent improvements in cat behavioral testing (Berkley,1970) and a simplified color vision test (Vis. Res.16:951,1976) encouraged us to return to this interesting question. Over a period of several weeks four cats were trained to discriminate blue vs grey or blue vs green. The cats' performance remained above chance but below criterion through manipulations of intensity, contrast, and pupil dilation. Increase in target size, however, improved the performance of three cats bringing two to the 90% correct level. Subsequent systematic variation in target size indicated that, for the cat, brightness but not color, is a feature of objects subtending a visual angle of 20 degrees or less. Supported by MH 28268-01.



1811 FUNCTIONAL DIFFERENTIATION OF THE LAMINAE OF THE LATERAL GENICULATE NUCLEUS OF THE RHESUS MONKEY. Joseph G. Malpeli and Peter H. Schiller*. Dept. Psych., M.I.T., Cambridge, MA 02139.

In that portion of the rhesus lateral geniculate nucleus (LGN) representing the central visual fields, there are 2 parvocellular and 1 magnocellular laminae receiving projections from each eye, for a total of 6 laminae. We have examined functional differences among these laminae in several male monkeys (Macaca mulatta) and have found a more pronounced laminar specialization than has been previously reported. Magnocellular neurons respond transiently to flashed stimuli, receive rapidly conducting retinal input, and have rapidly conducting axons. Cells of the parvocellular laminae have a relatively sustained response, receive slowly conducting retinal input, and have slowly conducting axons. The upper 2 parvocellular laminae (6 and 5) are generally heavily dominated by ON-center cells, while the lower 2 parvocellular laminae (4 and 3) are dominated by OFF-center cells. An exception to this rule are cells which respond vigorously to blue light (Wratten #47B). Virtually all give ON responses and are located in laminae 4 and 3. Thus, to a first approximation, color-opponent cells in laminae 6 and 5 are red or green OFF-center, while those in laminae 4 and 3 are red or green OFF-center, or blue ON-center. In the magnocellular laminae (2 and 1) ON- and OFF-center cells are present in roughly equal proportions and do not have color-opponent properties. The overall segregation of ON- and OFF-center cells in the parvocellular layers is complete enough to allow selective reduction or elimination of the dark-edge response of many neurons in striate cortex by microinjection of a local anesthetic into lamina 4 or 3.

1812 VISUAL TRACKING WITH SHORT-TERM REVERSED VISION DURING HEAD MOVEMENT: FUNCTION OF RETINAL IMAGE SLIP? G. Mandl and G. Melvill Jones, Aviat. Med. Res. Unit, McGill Univ.,, Montreal, Canada H3G 1Y6.

Head turning to, say, the left, evokes a vestibulo-ocular reflex (VOR) designed to drive both eyes, in a compensatory fashion, to the right. This reflex is normally capable of maintaining a stable retinal image of the visual world moving momentarily to the right relative to the head.

With optically reversed vision (left-right), however, head turning to the left will create an apparent motion of the visual world to the left. This results in conflicting vestibular and optokinetic drives to the oculomotor system, leading in turn to retinal image slip. It has been previously demonstrated that long-term visual reversal in normal light eventually leads to adaptive modification (gain and phase) of the VOR, with the apparent goal of minimizing retinal image slip.

We now report that, under stroboscopic illumination, subjects exposed to optically reversed vision for the first time, can almost immediately produce reversed smooth tracking eye movements in response to head rotation, in opposition to the prevailing vestibulo-ocular drive, and despite the absence of significant retinal image slip with this intermittent form of illumination.

Subjects wearing reversing prism goggles were seated, with heads fixed to a turntable, rotating either sinusoidally (velocity amplitudes 5,15 and 30°/sec, 0.17 Hz), or with a square wave velocity of amplitude 10°/sec, 0.1 Hz. Eye movements were recorded by dc EOG. After an initial control period in the dark, stroboscopic illumination (2 Hz) was suddenly switched on. Almost immediately, episodes of smooth tracking eye movements in the "reversed" (i.e. VOR opposing) direction could be recorded. In some cases, the first two light flashes were sufficient to induce reversed smooth pursuit eye movements during several 500 millisecond dark intervals between consecutive flashes.

Supported by the Canadian MRC.

1813 NEURAL MECHANISMS OF ORIENTATION DISCRIMINATION IN PRIMATE VISION. R. J. W. Mansfield, Steven F. Ronner* and Gordon E. Legge.* Dept. Psych., Harvard University, Cambridge, MA 02138.

The striate cortex in man and other primates plays an important role in fine pattern discrimination. Differential responses to oriented stimuli are a distinguishing feature of striate neurons in both man and Rhesus monkey. We have investigated the neural basis of orientation acuity by comparing the receptive field properties of striate neurons in the Rhesus monkey with the results of human psychophysical experiments.

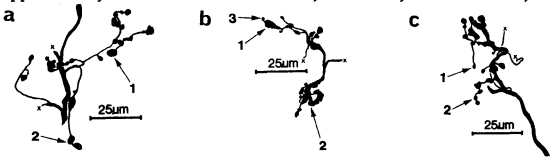
Receptive field properties were quantitatively assessed in 256 cortical units recorded in either foveal (0 - 2°) or parafoveal (4 - 7°) *projection regions of Area 17 in unanesthetized Rhesus* monkey. The majority of units recorded in the upper layers of the striate cortex (Layers II - IVb) possessed complex receptive fields with well-defined orientation selectivity. Orientation tuning curves were produced by varying in random order the angle of a stationary flashed bar or moving bar or slit. Parallel psychophysical experiments using human observers were conducted with similar pattern stimuli.

The following comparisons could be drawn: (1) the form of the human threshold curves for both foveal and parafoveal oriented targets was isomorphic to the corresponding population distribution functions for optimal orientations confirming a previous result (Mansfield, 1974, Science, 186, 1133); (2) for binocularly activated neurons, both the similarity of the orientation tuning curves and the low value for average orientation disparity, 5°, are consistent with the marked binocular interactions observed in the detection of oriented stimuli; (3) for the neural orientation tuning curve, half-bandwidth at half-amplitude was distributed in a unimodal log-Gaussian manner with median value close to that obtained for human orientation channels, 22°; (4) both neural and psychophysical orientation bandwidth were independent of optimal or adapting orientation; (5) both neural orientation tuning curves and the threshold curves defining human orientation channels were well-fit by theoretical functions calculated from a simple mathematical model based on sustained responses from non-oriented subunits.

These results support the hypothesis that the population response profile for neurons in Area 17 determines behavioral discriminability for oriented targets. (Supported by NSF grant BNS 75-08437 and NIH fellowship EY 05075.)

1814 MORPHOLOGY OF RETINO-GENICULATE AXONS AND THEIR TERMINALS IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. C.A. Mason* and J.A. Robson* (SPON: R.W. Guillery). Dept. Anatomy, Univ. Wisconsin, Madison, WI 53706

When retino-geniculate axons are diffusely filled with horseradish peroxidase (HRP), Golgi-like detail can be seen. Injections (0.2μl) of 50% HRP (Sigma, type VI) were made into the optic tract. After 24 hr survival and routine aldehyde fixation, brains were cut at 100μm on a Vibratome. Sections were soaked in 0.5% $CoCl_2$ in 0.1M Tris buffer (Adams, 1977, Neuroscience 2:141), and then incubated in DAB. Cobalt treatment changes the reaction product to blue-black. This procedure produces continuous filling of axons and their terminals, and it is our experience that it occurs only if the axons are cut. Axonal filling extends only about 1 cm from the injection site, and, as with the Golgi methods, not every axon is stained. Axons of varying diameters enter the LGN, and some are seen to be collaterals of axons in the optic tract. In laminae A and A1 axons are of large diameter and form terminal arrays aligned perpendicular to the laminae. These axons divide into several branches, and their terminal configurations are formed by swellings of several shapes and sizes, as shown below. Large swellings (7-10μm) are elongated and commonly crenulated; they occur singly (b1) or in the center of a cluster (b2). Medium-sized swellings (3-7μm) tend to be smoother and more spherical. They can be grouped around a large swelling in a cluster (b2), can occur at the end of fine axons (a1), or can form chains connected by fine axons (a2). Small swellings (1-2μm) can also occur at the end of (b3,c1), or as part of chains of (c2), fine axons. The C layers contain axons that are finer and run parallel to the laminae. These axons branch less frequently and give rise to swellings similar to the small swellings in A and A1. Axons in the medial interlaminar nucleus show no obvious alignment and resemble axons in the A layers both in terms of fiber diameter and shape of swellings. This labeling method reveals a far richer display of retino-geniculate terminations than do the Golgi techniques. Because myelinated segments are filled, axons and their branches can be traced over great distances, demonstrating that single axons form a variety of morphologically distinct terminal patterns.
(Supported by USPHS Grants NS11869, NS06662, and NS05407).



1815 TILT CONSTANCY MECHANISMS IN KITTEN VISUAL CORTEX. Jacqueline Metzler† and D. Nico Spinelli. Departments of Computer and Information Science and Psychology, University of Massachusetts, Amherst, MA 01003.

The visual world remains perceptually quite stable whenever we tilt our head. There is physiological evidence (Horn, et al., 1969, 1972; Spinelli, 1970; Denney and Adorjani, 1972) that in the cat this phenomenon of orientation or tilt constancy might result from changes in the orientation selectivity of a small percentage (about 6%) of the neurons in the visual cortex during head and body tilt. In an effort to test the effects of experience on the development of tilt constancy, eight kittens were reared under conditions of selective visual experience beginning at about 3-1/2 weeks of age. Each animal's total visual experience consisted of viewing two vertical bars with one eye and two horizontal bars with the other eye. In one eye the two bars were gravity-stabilized so that their orientation remained constant whenever the kitten rotated its head, while in the other eye the two bars were fixed so that they always maintained a constant orientation on the retina even when the kitten's head was tilted. Electrophysiological recordings of single units in visual cortex (area 17) were taken when the animals were between 12 and 20 weeks old. The receptive field of a cell was mapped when the animal was in the standard position, when tilted 23° clockwise or counterclockwise, and again in the standard position.

The receptive fields of 94 units were thoroughly analyzed and classified as either diffuse (55%) or elongated (45%). Units with elongated receptive fields responded to input from only one eye. Furthermore, when mapped in the standard position, the receptive field orientations were coincident with the bar pattern to which the eye had been exposed. When the kitten was rotated about its longitudinal axis, the orientation of those receptive fields that could be mapped only through the eye that had viewed the pattern having a fixed retinal orientation during development rotated by the same amount in the same direction; i.e., these receptive fields were not tilt constant. In contrast, the receptive field orientation of cells activated only by the eye that had been exposed to the gravity-stabilized pattern during development remained unchanged following body tilt; i.e., these receptive fields appeared to demonstrate tilt constancy. The results corroborate the findings that the receptive field properties of some cells in visual cortex can be modified, one of the modifying influences being head or body orientation. (Supported by NIMH Postdoctoral Fellowship 1 FO2 MH44282-01 to JM and NIMH Grant No. 7-R01 MH25329 to DNS.)
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1816 COLOR VISION MECHANISMS IN MONKEY STRIATE CORTEX. Charles R. Michael. Dept. of Physiology, Yale School of Medicine, New Haven, Conn. 06510.

Extracellular recordings were made with tungsten microelectrodes from 863 color-sensitive cells in the striate cortex of 26 rhesus monkeys. In area 17 there were four classes of color-coded neurons: concentric, simple, complex and hypercomplex. All four types responded only to monochromatic stimuli.

Cells with concentric receptive fields (137, 16%) had one red-green opponent-color system in the circular field center and the opposite organization in the annular surround. Simple color cells (131, 15%) had receptive fields consisting of a central rectangular strip containing one red-green opponent-color system and two antagonistic flanks with the reverse opponent-color arrangement. Complex color cells (382, 44%) responded only to moving bars or edges of monochromatic light. Hypercomplex color cells (213, 25%) similarly were excited only by moving monochromatic line stimuli but in this case they had to be limited in length at both ends.

Evidence from multiple-unit recordings has led to the following proposed synaptic connections: lateral geniculate afferents (two types) to concentric cells to simple units to complex neurons to hypercomplex cells.

Color units were arranged in vertical columns which extended through all six cortical layers. Within a column all cells were color-sensitive but varied in their individual chromatic properties, receptive-field organization, axis orientation and eye preference. The boundaries of the color columns were independent of those for the axis orientation and ocular dominance columns. Color columns were generally 100-200 μm wide but sometimes extended over greater distances. In a column color cells often had common or related chromatic properties, indicating evidence for a serial processing of wavelength information.

Complex and hypercomplex color cells were found in layers II, III, V and VI. Concentric and simple cells were confined almost entirely to layer IV. Most of the cells in IVC were concentric units.

All concentric cells and almost all simple units were monocular. The majority of complex and hypercomplex cells were binocularly activated.

Supported by NIH Grant EY 00568.

1817 CORTICOGENICULATE AXONS FROM AREA 17 AVOID GENICULATE SEGMENTS THAT RECEIVE AN ABNORMAL RETINAL INNERVATION IN SIAMESE CATS. V. M. Montero* and R. W. Guillery (SPON: C. N. Woolsey). Depts. of Neurophysiology and Anatomy, University of Wisconsin, Madison WI 53706.

Lamina A1 of the lateral geniculate nucleus of Siamese cats has a lateral segment that receives a normal uncrossed input from peripheral (20°-40°) parts of the temporal retina and a medial segment that receives an abnormal crossed input from more central (0°-20°) parts of the temporal retina. Injections of ^{3}H proline were made bilaterally in 4 adult "Midwestern" Siamese cats in area 17 on the medial wall of the lateral gyrus, 3 mm or 6 mm below the dorsal surface. At 3 mm, microelectrode recordings showed receptive fields about 5° into the contralateral visual field of the contralateral eye and no responses from the ipsilateral eye. At 6 mm, receptive fields were from the ipsilateral and contralateral eyes, 20° or more into the contralateral visual field. These two injection sites thus showed corticogeniculate projections to the abnormal and normal geniculate segments respectively. In 5 hemispheres, injections were made at 3 mm and these showed a characteristic dense distribution of label to layers A and C, but an almost complete absence of label in the interposed abnormal segment of layer A1. In contrast to this, 3 hemispheres with injections at 6 mm showed dense label in all geniculate layers, including the normally inervated segment of layer A1.

One of these Siamese cats had one eye sutured at 9 days of age, two had one eye removed (on the 8th and 19th postnatal day) and one was normal. Of the five hemispheres that showed avoidance of the abnormal segment of A1, one was from the normal cat, two from the lid sutured cat and two from the 19 day enucleate. The relative density of label in the geniculate layers was not affected by the deprivation or enucleation. In two normally pigmented cats, one with a unilateral (8 day) enucleation, the other with a (8 day) lid suture, bilateral cortical injections at 3 mm showed even, symmetrical label in all geniculate layers. These results suggest that the avoidance of the abnormal segment of layer A1 in Siamese cats is not dependent on visual experience. Corticogeniculate axons are present at birth in cats (Anker and Cragg, J.Comp.Neurol.154:29,'74), develop in anophthalmic mice (Kaiserman-Abramoff et al.,Neurosc.Abstract 1:102,'75) and appear to be unaltered by early enucleation or deprivation in our cats. The avoidance of the abnormal segment of layer A1 in Siamese cats must depend either upon the left:right reversal of connections or upon an intrinsic abnormal innervation pattern that distinguishes the abnormal from the normal retinogeniculate pathway.

1818 RESPONSE PROPERTIES OF CELLS IN THE NUCLEUS LATERALIS POSTERIOR OF THE CAT ASSOCIATED WITH VISUAL ORIENTATION. Brad C. Motter* and Donald B. Lindsley. Depts. of Psychology and Physiology, University of California, Los Angeles.

Extracellullar single unit recordings were obtained in the posterior extent of the nucleus lateralis posterior in cats trained to orient toward and fixate a small (0.75°) spot of light projected at various positions on a dimly illuminated (15 mcd/m^2) tangent screen located 60 cm. in front of the animal. During recording sessions the animal's head was fixed relative to the tangent screen and eye position was monitored with Ag-AgCl DC electrodes. Stimulus presentations were limited to those areas of the visual field in which each animal would maintain a visual fixation. A significant proportion of spontaneously active cells was found to tonically increase or decrease firing rate during fixation on the stimulus. Most cells in this category exhibited a gradient of responsiveness associated with the absolute position of the fixated spot within the visual field. No clear relationship between saccadic amplitude or direction and this gradient could be established. Firing rates of these cells were observed to return to background levels with eye movements away from the stimulus or at the offset of the two-second stimulus. Occassionally changes in the firing rates occurred with spontaneous fixations, i.e., when the stimulus spot was not present. A second group of cells showed an excitatory response to the onset of the stimulus spot, but were either non-responsive or phasically activated during the ensuing fixation. These cells had clear, but large receptive fields (based on initial eye position) usually including 2 or more receptive field quadrants. In nearly one half of the recorded cells there were no detectable changes in firing rate either to the presentation of the stationary spot or during fixation on that stimulus, however, changes in firing rates of some of these cells were observed during presentations of moving stimuli. Supported by USPHS grant NS-8552 and Grant Foundation supplementation.

1819 THE EFFECT OF ABLATION OF THE EXTRASTRIATE VISUAL SYSTEM ON VISUAL REVERSAL LEARNING IN THE RABBIT. E. Hazel Murphy and Eric Rappaport*.Dept. of Anat. The Med.Coll. of Pa.Phila.19129

The relative development of the extrastriate visual system (measured by the ratio of the pulvinar nucleus/ lateral geniculate nucleus) has been related to phylogenetic status. Greater development of the extrastriate visual system in mammals such as the tree shrew and bushbaby has been correlated with differentiation of visual areas of the temporal lobe and with the ability to form a learning set during successive reversals of a visual discrimination. Ablation of extrastriate visual cortical areas results in loss of this ability. In contrast, lesser development of the extrastriate visual system in mammals such as the rat and hedgehog has been correlated with lesser differentiation of visual areas of the temporal lobe and no learning set acquisition on daily reversal of a visual discrimination task. Because of this poor reversal learning performance, the effects of ablation of extrastriate visual cortex on reversal learning in these mammals have not been investigated. (Killackey et al. J.C. P.P. 1972, 81, 45., Masterton et al. Brain, Behav. and Evol. 1974, 10, 322.)

In rabbits, the pulvinar/ lateral geniculate ratio is similar to that of the rat and hedghog. However, we have previously shown that the occipital and temporal cortical areas of the rabbit contain several differentiated visual areas (Montero & Murphy,Anat. Rec. 1976). In the present study, we tested rabbits on tasks of brightness discrimination and reversals of this discrimination. The results showed that when normal rabbits were tested to criterion on each reversal (instead of being given daily reversals) then clear evidence for a learning set for visual reversals was obtained; the number of errors to criterion decreased significantly with each successive reversal. However, in rabbits, with ablation of extrastriate visual cortical areas in the occipital and temporal lobes, there was no evidence for a learning set; the number of errors to criterion on successive reversals remained constant. Normal and operated rabbits did not differ in their rate of learning the initial brightness discrimination. The results suggest that the extrastriate visual system of the rabbit has the same function in visual learning and learning set formation as it does in those mammals in which the system is, structurally, more highly developed.

Supported by USPHS Grant NS 13663.

1820 EARLY STRABISMUS PROTECT NEURONS IN KITTEN AREA 17 FROM THE EFFECTS OF MONOCULAR DEPRIVATION. Michael J. Mustari* and Max Cynader (Spon. I.A. Meinertzhagen). Department of Psychology; Dalhousie University; Halifax NS B3H 4J1.

It is now well established that rearing kittens with one eyelid sutured during early development results in the majority of single cells in Area 17 being driven only by stimuli presented to the non deprived eye. Hubel and Wiesel (1965) have reported that in kittens reared with artificial strabismus, the majority of cells were monocular but could be driven by either eye and that the separation between ocular dominance columns was more distinct in these cats then in normal cats. These data raise an interesting question, that is whether rearing kittens with artificial strabismus, thus separating ocular dominance columns, might produce a cortex that is less susceptable to the devestating effects of monocular deprivation. This study considers this question.

Two groups of kittens were used in this study. The first group (5 animals) were reared normally until day 45 (±3), monocularly sutured and recorded 8 days later. 200 units were studied in both hemispheres. The deprived eye lost functional connections with the visual cortex.

The second group of kittens were reared normally until day 15, and then were made strabismic by sectioning the right medial rectus muscle. They were kept in a normal visual environment until day 45 (±3) and then either the strabismic eye (11 animals) or normal eye (2 animals) was sutured closed for 8 days before recording from single cells (511 units) in Area 17. In the cortex contralateral to the monocularly deprived eye most neurons were monocular but driven by either eye. Regular shifts in ocular dominance occurred as a function of depth of the oblique electrode penetration. The ocular dominance distribution obtained from the cortex ipsitateral to the monocularly deprived eye in group two cats was very different and strongly resembled the ocular dominance distribution of the control cats. The results indicate that the cortex contralateral to the deprived eye in previously strabismic cats was resistent to the effects of monocular deprivation.

The data obtained in this study strongly argue that the physiological effects of monocular deprivation are medicated by competition between axons in right and left eye ocular dominance columns at the cortical level. The ipsilateral-contralateral differences found in the strabismic cats can best be accounted for by considering the anisotropy in size and distribution of ocular dominance columns found in normal cats. Correlative anatomical studies on these animals are in progress.

1821 CONVERGENCE AS A FUNCTION OF ECCENTRICITY IN THE RETINO-GENICULO-STRIATE SYSTEM OF THE OWL MONKEY. J. Myerson, P.B. Manis*, F.M. Miezin*, and J.M. Allman. Div. of Biology, Calif. Inst. of Tech., Pasadena, CA 91125.

In primates, the central portion of the visual field receives a greatly expanded representation in striate cortex. Investigators disagree, however, as to whether this is solely because of increased ganglion cell density near the center of the retina, i.e., "peripheral scaling," or whether the cortex provides additional "magnification." We compared the relative proportions of ganglion cells and striate cortical neurons for visual field zones of different eccentricities in the owl monkey. Calculations were based upon ganglion cell counts along horizontal and vertical meridians by Webb and Kaas (1976) and measurements of a three-dimensional brain model constructed on the basis of serial sections and electrophysiological mapping by Allman and Kaas (1971). The relative number of cells per degree^2 decreased more rapidly with increasing eccentricity in striate cortex than in the retinal ganglion cell layer. Functionally this suggests that in primates striate cortex is even more specialized than the retina for processing information concerning the center of the visual field. Anatomically, this means that the ratio of retinal ganglion cells to neurons in striate cortex increases with eccentricity. More specifically, the relationship between number of cells/degree^2 for corresponding portions of striate cortex (M_C) and the retinal ganglion cell layer (M_R) is describable by a power function: $M_C(\phi_1,\phi_2) = aM_R(\phi_1,\phi_2)^{**}2.35$. It follows that the ratio of ganglion cells (N_R) to striate neurons (N_C) is a power function of the area (A) per ganglion cell:

$$\frac{N_R(\phi_1,\phi_2)}{N_C(\phi_1,\phi_2)} = k\left|\frac{A(\phi_1,\phi_2)}{N_R(\phi_1,\phi_2)}\right|^{1.35}$$

Recent findings demonstrate that although some primate retinal ganglion cells send collaterals to the superior colliculus, all ganglion cells send axons to the lateral geniculate nucleus (LGN) (Bunt et al., 1975) and nearly all cells in the LGN project to striate cortex (Norden, 1974). Thus the preceding equation describes anatomical convergence in the retino-geniculo-striate system, i.e., the mapping of the retinal ganglion cell layer onto striate cortex. Our findings explain the results of recent autoradiographic studies (Kaas, Lin and Casagrande, 1976; Tigges, 1977). Following intraocular injection of tritiated proline, transneuronal labeling in foveal (dorsal) striate cortex is much less dense than in peripheral (calcarine) striate cortex because the ratio of ganglion cells/cortical neurons increases with eccentricity. (Supported by NIH Grants NS-12131 and NS-00178 and Sloan and Spencer Fellowships)

1822 BLINDNESS IN MONKEYS AFTER LESIONS OF NON-VISUAL CORTEX. Richard K. Nakamura and Mortimer Mishkin, NIMH, Bethesda, Md. 20014

Visual discrimination habits in monkeys are now known to depend not only on striate cortex, but also on secondary visual and associated limbic areas. In the present study we asked whether the visual and limbic systems acting together are sufficient for retention of such habits or whether any of the cortex outside these two systems also plays a significant role. To examine this question we restricted vision to a single hemisphere and then removed all the cortex in this hemisphere outside the visual and limbic systems. Since, in past studies, complete removal of sensorimotor cortex in the seeing hemisphere did not prevent visually guided motor behavior, we assumed that our planned lesions in this hemisphere would also spare such behavior sufficiently to permit an assessment of the role of non-visual, non-limbic cortex in retention of visual habits.

Four monkeys (2 rhesus and 2 cynomolgus) were trained on a visual pattern discrimination. The forebrain commissures and the right optic tract were then severed in each. Following retraining, three animals were given total left hemisphere cortical ablations sparing only: striate, prestriate, inferior temporal cortex; and medial temporal, ventral frontal, cingulate cortex. The fourth animal (a cynomolgus) received the same lesion except that the cortex anterior to the arcuate sulcus was spared.

Within two days of the cortical ablation all animals were able to right and feed themselves. Unexpectedly, however (though see Gazzaniga, Exp.Neurol, 16:289, 1966), it became clear at this point that all were behaviorally blind. Food could be found only by use of auditory, olfactory or tactile cues; fearful objects evoked no reaction; and unfamiliar environments were explored using only tactile cues. Two weeks after surgery these monkeys could not determine which of two foodwells contained exposed rewards without tactile searching. Twenty-five days after surgery one rhesus monkey was able to locate the exposed reward visually at just above chance; this ability was fully recovered after twenty days of further training, at which point the animal showed good first-day retention of the pattern discrimination habit though still exhibiting many of the earlier signs of blindness. This was the only monkey that could be tested. The other rhesus monkey showed no signs of recovery for 42 days and was sacrificed for histology; the lesions in this animal were as intended, and the primary visual pathways in the left hemisphere were found to be intact. The two cynomolgus monkeys have been kept alive for over 200 days and remain behaviorally blind.

We are currently testing whether this phenomenon is due to diaschisis of the visual system or to a visual-motor disconnection.

(Supported by NIMH Fellowship 5 F32 MH05273.)

1823 RELATION OF THE DELAYED GANGLION CELL RESPONSE AND THE E-WAVE TO ROD ACTIVITY IN THE FROG, RANA PIPIENS. Eric Newman* (SPON: L. S. Frishkopf). Dept. Biol., Research Lab of Electronic, M.I.T., Cambridge, Mass. 02139

The onset of both the delayed ganglion cell response and the e-wave have long latencies following light flashes presented to dark adapted eyes. An eye-cup preparation was employed to study the properties of these delayed responses and the mechanism generating the long delays. In this study, the e-wave appeared as a sharp, negative notch superimposed on a slow positive voltage recorded with an intra-retinal electrode referenced to the vitreous. It has latencies of from 2 sec to over 1 min, depending on flash intensity and adaptation state. Ganglion cell (types 3 and 4) activity was recorded while monitoring the e-wave response. A light flash produced a prolonged burst of activity following a silent period of as long as 1 min. Over a 5 decade range of flash intensities, producing delays of from 2 to 65 sec., ganglion cell activity occurred simultaneously with the e-wave. The latency to the start of the e-wave and the delayed ganglion cell response was roughly proportional to log flash intensity.

The extracellular mass receptor response was recorded in aspartate-treated eye-cups to determine the relation between receptor activity and the delayed retinal responses. Light flashes produced rod responses having prolonged plateaus, with onsets of decay from saturation delayed for up to 60 sec. The delay to the beginning of rod response decay for a given flash intensity corresponded closely to the delays seen to the onset of the e-wave and ganglion cell response. Retinal sensitivity, determined by a criterion local b-wave, was examined as a second measure of rod response. Retinal threshold remained elevated following flashes until the appearance of a sharp rod-cone break where the threshold fell steeply, indicating recovery of the rods from saturation. The onset of the e-wave occured concurrently with the beginning of the steep fall of retinal threshold. It is concluded that the delayed ganglion cell response and the e-wave both represent rod-driven events whose latencies are determined by the delay to the beginning of the rod response decay. (Supported by NIH training grant 5 T01 EY00090 and Bell Laboratories)

1824 A QUANTITATIVE EM INVESTIGATION OF CHANGES IN THE SYNAPTIC ORGANIZATION OF THE OPTIC TECTUM FOLLOWING ENUCLEATION IN XENOPUS. J.J. Norden, A.-J.C. Ostberg*, and J.A. Freeman. Vanderbilt Univ., Nashville, TN and NIMR and University College, London, England.

The morphological changes which occur in synapses following denervation are of considerable interest inasmuch as they may reveal important aspects of underlying mechanisms of synaptogenesis. Of particular interest is whether denervation can induce sprouting of other fibers which project to the region and if so, whether these fibers innervate vacated postsynaptic sites or induce new specializations.

In the present study, the time course and changes in synaptic organization which occur following denervation were examined quantitatively in the optic tectum of Stage 66 Xenopus using EM at times ranging from 12 hrs to 6 mos following unilateral enucleation. For each animal, the total number of synapses were counted in 10 grid squares at symmetric loci in the superficial tectal layers. Synapses were classified into 4 types ranging from normal to late degenerating. The number of vacant sites or postsynaptic specializations unopposed by a presynaptic element with clustered vesicles and the amount of glial and axonal debris were also counted.

Preliminary results indicate that by 24 hrs following enucleation over 20% of the synapses in the denervated tectum are undergoing degeneration. There is little difference between the denervated and control sides in the number of vacant sites or amount of debris. By 4 days, nearly 40% of the total number of synapses are degenerating and there is a dramatic increase in the amount of debris. The most significant finding at 4 days, however, is a large decrease in the total number of synapses without a significant increase in the number of vacant sites on the denervated side. The presence of pre- and postsynaptic elements still in contact within glial cells at this time suggests that both elements are removed by gliosis, leaving few vacant postsynaptic specializations. These findings suggest that following enucleation in Stage 66 Xenopus, fibers from the diencephalon and contralateral tectum which also synapse in the superficial tectal layers do not sprout to occupy postsynaptic sites vacated by optic nerve fibers. Any newly formed synapses would require the de novo synthesis of postsynaptic specializations.

1825 HOW DO VISUAL CELLS SYNTHESIZE RESPONSES TO MOVING LIGHT STIMULI? J. Outerbridge*, G. Mandl and M. Gorayeb*. (SPON: R. Capek) Aviat. Med. Res. Unit and BioMed. Engrg. Unit, McGill Univ., Montreal, Canada H3G 1Y6.

In an attempt to answer this question, the present experiments examined the responses of 3 cells to (1) spatial sequences of identical stimuli delivered successively to several non-adjacent locations within the central activating region; and (2) temporal sequences of two stimuli, delivered at varying inter-stimulus intervals to individual locations within the activating region. METHODS. Extracellular microelectrode records were obtained from visual cells in the intermediate and deeper layers of the superior colliculi of acute, unanaesthetized cats whose brainstems had been transected at the pretrigeminal level. To stimulate spatial sequences of non-adjacent receptive field regions, an opaque mask containing up to 5 narrow slits (2x40mm), spaced some 2° apart, was placed over the receptive field. A single light-dark border, moving across the slits at uniform velocities of 4-256°/sec, produced step-like unidirectional changes of light intensity (dimming) at successive, fixed locations. Temporal sequences of dimming stimuli, confined to individual locations, were produced by using a receptive field mask with a single slit. A two-step sequence of light intensity (double borders, white-grey, grey-black) was moved across the stationary slit, at a fixed velocity of 320°/sec, but with varying spacing between the two borders. Cell responses were evaluated using average response histograms. RESULTS. To predict a cell's response to spatial or temporal sequences of stimuli, the time course of excitability change due to an individual stimulus must be known. Owing to a cell's firing threshold, this could not be obtained from observed responses to single border stimuli. However, a complete picture of such excitability changes could be synthesized from experimentally-observed responses to spatial sequences of single border stimuli. Linear superposition of the synthesized excitability changes appeared to account for the cells' responses to spatial sequences of single border stimuli, but not to temporal sequences of double border stimuli.

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1826 NEURONAL CIRCUITS WITH "PRESYNAPTIC DENDRITES" IN DORSAL LATERAL GENICULATE NUCLEUS (LGNd) OF MONKEYS. Pedro Pasik, Tauba Pasik and József Hámori*. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029, and 1st Dept. Anat., Semmelweis Univ. Med. Sch., 1450 Budapest, Hungary.

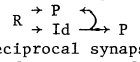
A prominent feature of the synaptic organization of the LGNd is the presence of "presynaptic dendrites" exhibiting both presynaptic and postsynaptic sites. The majority of these elements are processes of Golgi type II interneurons. They enter in synaptic relations with retinal (R) or cortical (C) axon terminals, dendrites of projective neurons (P), other interneuron dendrites (Id) or axons (Ia), and still other elements of undetermined origin. The present study has used the technique of serial sections of the LGNd from mature monkeys (M. mulatta) to analyze the various synaptic arrangements with Id participation. Three basic prototypes were found, namely triadic, serial and reciprocal synapses. In the triad, the Id element is postsynaptic to element #1 and presynaptic to element #3 which is also postsynaptic to element #1. Since element #1 and #3 can be R, C, P, Id or Ia in nature, 8 different kinds of triads involving these profiles may exist in the LGNd. So far, 4 of them have been found as follows:

The triad with retinal participation is by far the most common. In the serial synapses, the Id element is postsynaptic to element #1, and presynaptic to element #3. Seven of the 8 possible combinations have been already identified, namely:

R → Id → P R → Id → Id C → Id → P C → Id → Id

Ia → Id → P Ia → Id → Id Id → Id → P

In the reciprocal synapses, the Id element is presynaptic and postsynaptic to another element of similar nature. This type, although rare, is also present in the LGNd: Id ⇄ Id. In addition to these basic prototype circuits, there are also linkages at a distance between, for example, a triadic and a serial arrangement, the linking element being the same Id profile. The most frequent of these combinations is a triad with retinal input where the Id profile is also presynaptic to a distant P element, probably belonging to a different projective neuron:



The existence of triadic, serial and reciprocal synapses offers multiple possibilities for the processing of sensory information dealing with both spatial and temporal properties of stimuli. Additional knowledge is needed, however, on the operational features of the interneurons as well as a direct physiologic evidence for their assumed inhibitory nature. Aided by N.I.M.H. Grant MH-02261.

1827 VISION OF CATS REARED IN STROBOSCOPIC ILLUMINATION. Tatiana Pasternak, William H. Merigan and John Lott Brown. Center for Visual Science, University of Rochester, Rochester, NY 14627.

Cats were reared from birth to at least 12 mo. in a room illuminated only by a short flash (3 μsec) every 1.5 sec. This environment provided patterned visual stimulation but precluded the perception of movement. Single unit studies of these animals have shown normal responses in the optic tract but some reduction in orientation selectivity and highly significant reduction in directional selectivity of cortical cells (Levitt, Emerson, Brown, 1977). Behaviorally, upon removal from the stroboscopic environment, the cats showed no visual following, no startle to a looming object, poor visually guided placing and severe deficits in visually guided reaching (serrated edge test). The cats locomoted on bent legs, bumped into barriers in an obstacle course and would not jump down from a height of 30 cm. Optokinetic nystagmus was present. After several months in a normal environment, the animals showed improvement in some tests, but they remained deficient in visually guided reaching, showed no startle response to a looming object and did not jump normally.

Quantitative behavioral measurements of the spatial modulation transfer function have been undertaken on three experimental and three control cats following at least two months of adaptation of the experimental animals to a normal environment. In a two-alternative forced-choice procedure, the cats are presented with a vertical sinusoidal grating and a blank field of equal mean luminance displayed on two CRTs. The position of the grating is varied at random from left to right on successive trials. The cats are rewarded for a nose pressing response towards the grating. Results indicate a reduction in the high frequency cut-off and reduced sensitivity to intermediate spatial frequencies for the strobe-reared cats as compared to the normal animals. (Supported by NIH Grant EY 00680-05)

1828 LOCAL NOREPINEPHRINE PERFUSION AND EFFECTS OF MONOCULAR DEPRIVATION IN 6-OHDA-TREATED KITTENS. John D. Pettigrew* and Takuji Kasamatsu. Beckman Labs. of Behav. Biol., Calif. Inst. of Tech. Pasadena, CA 91125.

We have adopted a regime of 6-OHDA treatment which produces significant catecholamine depletion in the cortex of kittens after intraventricular administration. The doses of 6-OHDA required are proportionately much larger than those which appear to be adequate for rodents. In addition, these doses do not produce significant depletion of catecholamines from brainstem regions.

As described previously, we find that visual cortical neurons in 6-OHDA-treated kittens do not respond in the usual way by changing ocular dominance after monocular occlusion. Recordings from normally-reared kittens treated with 6-OHDA following a period of monocular deprivation are consistent with the hypothesis that cortical plasticity, as measured by the change in ocular dominance, is dependent upon the presence of catecholamines.

To test this hypothesis further we have developed a technique of local cortical perfusion of norepinephrine from a fine cannula attached to an osmotic mini-pump (Alzet). Virtually complete ocular dominance shifts are observed in 6-OHDA-treated kittens amongst those neurons in the cortical region close to the infusion site following one week of monocular closure simultaneous with one week's delivery at 1 μl/hr of a solution containing 0.4% ascorbate in saline (pH=3) whose initial concentration of norepinephrine was 0.1 μg/μl. Such ocular dominance shifts appeared to be confined to cortex which was reached by norepinephrine. High proportions of binocular neurons, without a bias for one eye, were observed in the opposite visual cortex of the same kittens, in the regions surrounding control cannulation sites which had been perfused with the acidic vehicle solution alone.

(Supported by Spencer Foundation and NIH Grant MH25852).

1829 CALCIUM SPIKES EVOKED IN TURTLE CONES BY PERIPHERAL LIGHT STIMULATION. M.Piccolino* and H.M.Gerschenfeld*(SPON:E.Marder). Lab.Neurobiol., Ecole Normale Supérieure,75005 Paris,France.

Cones of the turtle Pseudemys scripta elegans, when illuminated with a bright light on the periphery of their receptor field during light stimulation of its center, show a synaptic depolarization (Baylor, Fuortes and O'Bryan, J.Physiol.,214, 265,1971) which in some cases may give rise to a spike-like response of 10-20 mV (Fuortes,Schwartz and Simon, J.Physiol. 234,199,1973; O'Bryan, J.Physiol. 235,207,1973).

We have found that injecting long pulses of outward current the feed-back synaptic depolarization can be converted to a 15-30 mV spike. Sr^{++} ions (4-6 mM) facilitate the appearance of these spikes either with or without current injection.

In many cones the feed-back depolarization disappears spontaneously or is never observed. In this case, peripheral light stimuli, or the proper combination of central or peripheral stimulation can evoke spikes of 30-45 mV after prolonged application of Sr^{++} or Ba^{++} ions (6 mM). 4-aminopyridine(4-AP) facilitates the appearance of the Sr^{++}-induced spikes. The application of Ba^{++} or of both Sr^{++} and 4-AP evokes Ca-spikes appearing at the off of the cone responses, similar to those described in the toad rods (Fain,Quandt and Gerschenfeld,ARVO Meeting,1977).Atropine (5 mM), which hyperpolarizes the horizontal cells and block their responses, as well as glutamate ions (50 mM), which depolarize the horizontal cells and block their responses, both block preferentially the synaptically evoked spikes. These are not affected by 10 μM tetrodotoxin, but are blocked by Co^{++} ions and D-600 (0.5 mM).

These experiments suggest that stimulation of the periphery of the receptor field of turtle cones can facilitate through a synaptic mechanism a regenerative Ca^{++}-conductance.
(Supported by grants from C.N.R.S. and D.G.R.S.T., France)

1830 CLASSIFICATION OF RECEPTIVE FIELD PROPERTIES OF CELLS IN THE VISUAL CORTEX. Karl H. Pribram, Maryse Lassonde and Maurice Ptito. Psychology, Stanford Univ., Stanford, CA 94305

Quantitative analyses of receptive field properties of cells in the visual cortex were performed using spots, single lines, double lines and multiple lines (gratings). Results were classified on the basis of whether the properties examined varied independently or covaried. Independently varying properties were found to delineate stimulus parameters while the covarying properties described the cells' response. However, an intermediate level that defines the interactive dimensions of the dendritic field of the cell was distinguished because two classes of covariation were identified. The interactive dimensions were examined especially in the two-line experiments. The results of these experiments showed that simple receptive fields are characterized by definitely separate "zones" of excitation and inhibition with a neutral region between. Complex receptive fields, on the other hand, display inhibitory interactions over a wider region. The independently varying properties were especially clearly delineated by exposure to gratings. First, these characteristics confirm the stimulus orientation and direction of movement selectivity of cells in the visual cortex as described by single and double line experiments. Second, an additional stimulus parameter is added: spatial frequency. There is no covariation of spatial frequency selectivity in our results with any other parameter investigated, nor is there covariation between the bandwidth of the spatial frequency selectivity and these other parameters. Additionally, two classes of covarying interactive properties of the receptive field were distinguished by the use of stationary sine wave gratings when phase angle was reversed and by sine wave gratings drifted across the field in the preferred orientation and direction. Enroth-Cugell and Robson (1966) using these techniques described two populations of retinal ganglion cells which they labeled "X" and "Y". In our experiments, the same criteria defined populations which were practically coordinate with those defined by hand held and computer controlled single-line stimuli as "simple" and "complex" (only 2 out of 47 cells failed to match). Most investigators have assumed an equivalence between sustained response and "X" characteristics and between transient response and "Y" characteristics and between transient response and "Y" characteristics. Our results clearly show that the X-Y and sustained-transient classes are orthogonal to each other and not coordinate. Our data suggest further that the sustained/transient classification reflects basic output (axonal) properties of visual cells while the X-Y, simple-complex classification appears to reflect input (dendritic) properties.

1831 EFFECT OF PRENATAL UNILATERAL EYE ENUCLEATION ON THE FORMATION OF LAYERS AND RETINAL CONNECTIONS IN THE DORSAL LATERAL GENICULATE NUCLEUS (LGd) OF THE RHESUS MONKEY. Pasko Rakic, Dept. of Neuropathology, Harvard Med. Sch. and Dept. of Neuroscience, Children's Hospital Medical Center, Boston, Mass. 02115

Single eye enucleations were performed in two fetal monkeys exteriorized from the uterus at E63 (63rd embryonic day) and E91. The fetuses were replaced in utero and carried to full term (165 days). At 3 months of age, the remaining eye of each monkey was injected with a mixture of H^3-proline and H^3-fucose. Fourteen days later, the animals were fixed by perfusion and their brains processed for light microscopic, autoradiographic and electron microscopic analysis.

In the 3-month-old monkey in which one eye was enucleated at E63, the LGd is positioned normally and retains its usual size and "knee-like" shape, but it is totally devoid of layers. Therefore, the presence of retinal axons from both eyes around or after E63 may be essential for the development of LGd layers. In the 3-month-old monkey in which one eye was enucleated at E91, LGd is slightly smaller and discernible layers emerge only at the caudal pole of the nucleus. This incipient lamination is comparable to that attained in the fetus at the time of eye removal. Thus, even after being initiated, lamination does not proceed to completion if one eye is absent.

In each monkey, radioactive tracers are transported equally to both LGd's. In the animal enucleated at E63, grains are distributed uniformly over the entire nucleus; thus even neurons situated at the periphery, which normally receive input from the contralateral eye are surrounded by ipsilateral projections. Electron microscopy displays an abundance of retinal synapses in these areas which would normally be occupied by terminals from the enucleated eye. Thus, retinal axons form synapses with LGd neurons which they do not contact normally. In the monkey enucleated at E91, the presence of small, irregular and less densely labeled territories at the posterior moiety of the LGd suggests that the separation of fibers from the two eyes has stopped at the level achieved at the time one eye had been removed. Since projections from the two eyes initially overlap in the fetal monkey (Rakic, P., Nature, 1976, 261: 467) terminals from the remaining eye do not have to invade new neuronal territories. Rather, they may fail to retract to monocular domains.

On the basis of these experiments, it appears that both the development of laminae as well as the segregation of afferent connections in the primate LGd depend on prenatal competition between retinal projections from the two eyes.
(Supported by NIH Grant NS11233).

1832 FUNCTIONAL ANALYSIS OF GO-NO GO RELATED (ATTENTION) UNITS IN THE ROSTRAL BRAINSTEM. Constance L. Ray* (SPON: Allan F. Mirsky). Div. Psychiatry, Sch. Med., Boston University, Boston, MA 02118

Bakay Pragay and Mirsky (Neurosci. Abstr. #1556, 1976) used the Evarts' method to analyze neuronal correlates of performance on a go-no go visual discrimination task. Using rhesus monkeys, most of the rostral brainstem was explored. The monkeys learned to press a 'hold' button for two seconds. Then, either a red or a green light appeared on another response button above the hold button. If the light was red (go), the monkey had to lift its hand from the hold button and press the upper response button within one second to receive a juice reward. If the light was green (no go), the animal was reinforced for continuing to hold for 1 second. Many of the task related units were related to parallel events in both types of trial, i.e. 1) the onset of the cue stimuli and 2) the offset of the cue stimuli/delivery of the reinforcers.

In this work these task related changes in firing rate were studied to determine their relation to 1) the physical sensory configuration of the event, 2) the behavioral significance of the event and 3) event-evoked eye movements. When a task related unit was detected, it was first recorded during the task. Then with the task off, a series of cue stimuli (stimulus only condition) and a series of juice reinforcers (reinforcement only condition) were presented separately to the animal. (The animal's responses in the stimulus only condition had been extinguished previously.) Stereotyped saccadic eye movements were monitored continuously with Beckman minature electrodes.

The units which displayed peri-stimulus on responses during the task differed in their responsiveness to the physically identical stimuli presented during the stimulus only condition. Some units (type A) continued to respond to the stimuli in both contexts, suggesting a relation to the physical qualities of the stimuli. Other units (type B) responded to the stimuli only during the task. The B units which tended to be found in non-visual areas may be related to the reward value of the event. The peri-reinforcement responses could be due to the offset of the stimuli or to the delivery of the reinforcers; these occur almost simultaneously. We have been able to identify whether a given response is due to one or both of these events.

Analysis of EOG as well as unit recordings from the oculomotor complex during task performance suggest that eye movement evoked changes seldom occur in parallel in both trials. In general task evoked saccades occur most regularly in the positive trial during the go response.

1833 A REDEFINITION OF PULVINAR SUBDIVISIONS IN THE MACAQUE MONKEY: EVIDENCE FOR THREE DISTINCT SUBREGIONS WITHIN CLASSICALLY DEFINED LATERAL PULVINAR. Michael Rezak and L. A. Benevento. College of Medicine, University of Illinois at the Medical Center, Chicago, Illinois 60680.

Traditionally the lateral pulvinar (PL) of the macaque thalamus has been considered a single anatomical and functional entity. Our previous and ongoing autoradiographic studies (Brain Res., '75; '76; '77 and Anat. Rec., '77) have shown that this traditional view is incorrect and that this may be a source of the discrepancies among recent anatomical reports. Thus, as a result of placing discrete microinjections (.1µl) of tritiated leucine and proline along the dorsal-ventral and rostral-caudal axes of classically defined PL of 15 macaque monkeys and observing the cortical projection targets we propose to redefine PL in terms of 3 subregions i.e., PL_α, PL_β, and PL_γ. PL_α forms a narrow band approximately 2-3mm wide surrounding the inferior pulvinar dorsally, ventrally and laterally. PL_α contains a precise map of the visual hemifield and projects this map to layers I-II of striate cortex and layers III, IV and I of prestriate cortex. PL_β lies dorsal to PL_α and has as its cortical target layers III, IV and I of the parietal lobe (areas 5 and 7) as well as dorsal portions of the temporal lobe. Finally, the lateral portion of the caudal pole of the pulvinar forms PL_γ and has as its cortical projection site layers III, IV and I of inferotemporal cortex (areas 20 and 21). Neither PL_β nor PL_γ project to striate cortex. Based on the results of injections of tritiated amino acids into occipital cortex we have found that all three subregions of PL receive input from these visual cortical areas, however, only PL_α receives an input which is clearly organized with respect to visual hemifield representation. In addition it should be noted that PL_β is the target of projections from retinorecipient portions of the pretectum (Brain Res., '77) thus forming an important link in visuomotor function. (Supported by NSF Grant BMS 75 07349)

1834 VISUAL RESPONSES DURING SACCADIC EYE MOVEMENT: A COROLLARY DISCHARGE TO SUPERIOR COLLICULUS. Barry J. Richmond and Robert H. Wurtz. Lab of Neurobiology, National Institute of Mental Health, Bethesda, Md. 20014

Some cells in the superficial layers of the monkey (Macaca mulatta) superior colliculus respond to rapid stimulus movement across the stationary receptive field but fail to respond when a saccadic eye movement sweeps the stimulus across the receptive field. The differentiation between real stimulus movement and self-induced stimulus movement resulting from the eye movement is not due to visual stimulation since it persists when the background light is reduced. In addition a suppression of discharge rate occurs following eye movements made in total darkness indicating that the suppression is not visual in origin (Robinson and Wurtz, 1976). To determine whether this extra-visual input was dependent on the movement of the eye (a proprioceptive input or mechanical shearing of the retina) or resulted from input from the oculomotor system (a corollary discharge) we paralyzed the eyes in two monkeys and used the suppression of the discharge rate during attempted spontaneous eye movements as an indicator of extra-retinal input. Retrobulbar injections of 1% xylocaine produced complete paralysis of extra-ocular movements lasting 15 to 20 minutes; no eye movement greater than 3 minutes of arc (the smallest we could resolve) was observed during the period of complete paralysis. Attempted eye movements by the monkey were indicated by integrated multiple unit activity recorded from a chronically implanted macroelectrode in the oculomotor nucleus. The discharge of 17 cells which showed the suppression of background discharge with normal eye movement continued to show the suppression during the complete paralysis. For several cells, the suppression was observed before, during, and after the period of paralysis. Since the suppression, and therefore the extra-retinal input, was just as pronounced when the proprioception and shearing factors were eliminated, the elimination of visual response must result from a potent corollary discharge to the monkey superior colliculus.

1835 FUNCTIONAL PROPERTIES OF POSTERIOR PARIETAL CORTEX OF THE MONKEY. I. SENSORY RESPONSES. David Lee Robinson and Michael E. Goldberg. Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20014.

On the basis of clinical observations and experimental studies, posterior parietal cortex has been thought to associate information from the visual and somatosensory modalities with behavior, such as limb movements and eye movements. By recording from several hundred movement-related single neurons in areas 5 and 7 of three awake, trained monkeys, we have found that such cells respond to passive visual and/or somatosensory stimulation. In addition, the responses of some of these cells can be modulated by behavioral conditions which are the subject of the subsequent abstract.

Passive visual stimuli were presented while the monkey fixated a spot of light on a tangent screen; the animal was not required to make any movement toward the visual stimuli. Posterior parietal cells have very large receptive fields. These frequently include a whole visual quadrant; on occasion the fields include a whole hemifield and can be bilateral. These visual fields can include the fovea. Some cells have tonic responses to stimuli whereas other respond phasically; the activity of these cells during eye movement tasks is determined by these visual properties, i.e., tonic cells discharge during fixations and tracking eye movements, phasic cells discharge with saccadic eye movements. Parietal cells are not sensitive to the orientation of stationary stimuli. Most parietal cells respond very well to large stimuli and less well to small spots of light. The visual responses of these cells can be modulated by the intensity of the stimulus. Many cells respond equally well to all directions of stimulus movement although a subset of neurons are directionally selective.

Passive somatosensory stimuli were delivered while the animal sat quietly in his chair and could not see his limbs. Somatic receptive fields of posterior parietal neurons are similar to the visual receptive fields in that they are quite large. Somatic receptive fields frequently include whole limbs, either the forelimb or hindlimb. Some cells respond to passive somatosensory stimulation of the ipsilateral or contralateral limb while others respond best to simultaneous bilateral stimulation. Although many posterior parietal neurons are excited by somatic stimulation, some can have their spontaneous activity inhibited.

We believe that our data support the association hypothesis for the function of posterior parietal cortex, and we suggest that this area, in conjunction with other parts of the visual and somatosensory systems, helps to determine a sensory environment in which movement might be appropriate.

1836 THE SYNAPTIC ORGANIZATION OF TERMINALS TRACED FROM INDIVIDUAL LABELED RETINO-GENICULATE AXONS IN THE CAT. J.A. Robson* and C.A. Mason* (SPON: A.W. Clark). Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706

The synaptic organization of different terminals of individual retino-geniculate axons labeled with horseradish peroxidase (HRP) was studied in the dorsal lateral geniculate nucleus. HRP was injected into the optic tract and the tissue was prepared as described by Mason and Robson (this volume). However, sections were not soaked in $CoCl_2$ since this appears to detract from the quality of tissue preservation. After the sections were incubated in diaminobenzidine, they were osmicated and embedded flat between plastic slides. Individual axons in lamina A were drawn with a camera lucida and 5µm sections were cut through the drawn axons. After the labeled axons in the 5µm sections were matched to the drawing, the sections were thin-sectioned. Ultrastructural examination revealed that labeled axons contain an electron-dense reaction product distributed throughout the cytoplasm. This reaction product tends to coat membranes and consequently, the cytoplasmic surfaces of synaptic vesicles and mitochondria are densely outlined. However, the synaptic vesicles are seen to be round and the mitochondria are pale with widely spaced cisternae. Specialized synaptic zones are also discernable and the labeled terminals have never been seen to be postsynaptic. Thus, all of the labeled terminals are classifiable as RLP-terminals, previously identified as arising from retino-geniculate axons. One of the most striking results of this study is the variation in the number of synaptic contacts made by different terminals of the same axon. That is, a terminal may be small and contact only a few process (1-3) while another terminal of the same axon may be large and contact many processes (20 or more). The largest terminals (7-10µm) are always the central process in a glomerulus containing numerous postsynaptic profiles. These profiles often indent the central, labeled terminal giving it a crenulated appearance also seen light microscopically (Mason and Robson, this volume). Smaller, intermediate sized terminals (3-7µm) often participate in glomeruli but may also form solitary contacts onto dendritic shafts. The smallest terminals (1-2µm) are round and are most often found to contact dendritic shafts outside glomeruli. Furthermore, several terminals from one axon may contact the same dendrite and a single dendritic stem may be contacted by some labeled and some unlabeled retino-geniculate terminals. This method provides a new way of identifying retino-geniculate axons and their terminals and demonstrates that a surprising number of these terminals form synaptic contacts outside of glomeruli. (Supported by USPHS Grants NS11869, NS06662 and NS05407).

1837 GENICULATE PROJECTIONS TO STRIATE CORTEX (AREA 17) IN THE SQUIRREL MONKEY AS DEMONSTRATED BY TRANSNEURONAL AUTORADIOGRAPHY. M. H. Rowe, M. Rezak and L. A. Benevento. College of Medicine, University of Illinois Medical Center, Chicago, Illinois 60680.

The pattern of geniculo-striate projections has been examined in the squirrel monkey by means of transsynaptic autoradiography, and compared to the patterns reported in certain other primates. 1 mCi of tritiated fucose and 1 mCi of tritiated proline were injected into the vitreous chamber of the left eye of one adult monkey. The animal survived 3 weeks and the brain sections were exposed to Kodak NTB-2 emulsion for 6 weeks. First-order grains were visible to the naked eye in the superior colliculus, pretectum and dorsal lateral geniculate nucleus (DLG). With the aid of darkfield microscopy second-order grains were readily apparent in structures such as the inferior pulvinar and striate cortex, presumably due to transsynaptic transport from the superior colliculus and DLG, respectively. No grains were apparent in area 18, suggesting that the DLG does not project to this cortical area. The grains in area 17 were seen only in layer IV and ended abruptly at the border between areas 17 and 18. In area 17, layer IV can be cytoarchitecturally subdivided (as in the macaque) into 3 sublayers i.e., IVa, IVb and IVc. In striate cortex of both hemispheres the grain density was highest in layer IVc. Both layer IVa and the lower half of IVb contained a moderate amount of grains, while the upper half of layer IVb was relatively free of grains. Thus, in this respect the laminar distribution of grains was essentially identical to that seen in the macaque monkey. However, at these survival and exposure times grains were apparently present throughout the horizontal extent of layer IV in both hemispheres. The only difference between the two striate cortices was that the overall density seemed slightly lower on the ipsilateral side. Although there were some horizontal variations in grain density apparent in layer IV on both sides, these variations did not seem to form any systematic pattern. Whether or not these variations represent a relatively undeveloped system of ocular dominance columns like those reported in the cat, is not yet clear. (Supported by NSF Grants BNS 75-07349 and 75-17890)

1838 RETINAL DEVELOPMENT IN THE LAMPREY, PETROMYZON MARINUS. K. Rubinson, H. Ripps, P. Witkovsky and M.C. Kennedy. Dept. Physiol. and Biophysics, NYU Sch. Med., Dept. Anat., SUNY at Stony Brook, and Dept. Biol., NYU, New York, NY 10016.

Morphological and physiological studies were conducted on retinas of larval and transforming sea lampreys. Electrical activity, spectral absorption measurements and tissue for electron microscopic observation were obtained from the same retinas.

In larvae less than 1 year old, the neural retina is divided into nuclear zones, but plexiform layers and short, receptoral outer segments are only visible in a small region surrounding the optic disc. Nevertheless, in these animals, no ERG can be recorded and absorbance spectroscopy fails to demonstrate the presence of any photolabile pigment. This appearance of the retina, compacted, yet differentiated centrally, is seen in larvae of all stages. It is connected with the brain and the central projections have been described.

At stage IV of transformation, outer segments and plexiform zones are seen throughout the retina. The photoreceptors are of 2 types, long and short, but both possess pedicle-like terminations as demonstrated by Golgi impregnation. The outer segments, throughout the retina, are longer than those of the larvae and contain well-ordered discs. All fully-impregnated bipolar cells possess Landoldt's clubs.

Stage V is similar to stage IV with a slight increase in outer segment length. ERG responses can be evoked, as they can be in stage IV, and absorbance spectroscopy of the isolated retina reveals the presence of a photolabile pigment whose absorption maximum is at 510 nanometers.

The inability to measure either photopigment or electrical responses in larval stages whose retinas possess substantial numbers of outer segments suggests that the presence of visual pigment is not essential to the maintenance of disc integrity.

1839 BRIEF PERIODS OF BINOCULAR PARALYSIS IN THE ADULT CAT PRODUCE REDUCTIONS IN ENCOUNTER RATES FOR SELECTED CELLS IN THE LATERAL GENICULATE NUCLEUS. W.L. Salinger, P.R. Wilkerson*, and M.G. MacAvoy*. Psych. Dept., Univ. N. Carolina, Greensboro, 27412.

Binocular paralysis produces substantial changes in the electrophysiology of the lateral geniculate nucleus (LGN) in the adult cat. These effects do not appear to arise from accidental surgical trauma, since in producing the paralysis, neither the orbit nor the meninges are penetrated, and the bone covering over the optic nerve and optic chiasm remained intact. The nature and time course of the effects of binocular paralysis therefore should reflect functional changes in the visual system.

To assess such functional change, single units were recorded from the LGNs of adult cats which had bilateral transections of cranial nerves III, IV, and VI for four days or less at the time of recording. The latency of response to optic chiasm shock (OX latency) was measured for each unit. Within four days following paralysis, the LGN contained significantly fewer cells with OX latencies in the X-cell range than is the case in normal animals.

This rapid change in the electrophysiology of the LGN after binocular paralysis may have parallels in the rapid "release" of cortical cells from the effects of monocular deprivation; produced in the adult either by ennucleation of the non-deprived eye, (Kratz, Spear, and Smith, J. Neurophys., 1976) or by the administration of bicculline or ammonium acetate (Duffy, Burchfiel, and Snodgrass, Neuroscience Abst., 1976). Whether or not these parallels obtain, the effects of binocular paralysis reaffirm that the organization of the adult visual system is extremely responsive to modifying environmental influences. Moreover, the extent of this continuing sensitivity exceeds what one would expect based on earlier work on sensitive periods for development and maintenance of orientation selectivity and ocular dominance properties of cortical cells.

1840 PYRAMIDAL CELLS IN THE OPTIC TECTUM OF A TURTLE, PSEUDEMYS SCRIPTA ELEGANS. P.B. Schechter and P.S. Ulinski. Departments of Ophthalmology and Anatomy, University of Chicago, Chicago, Ill. 60637.

Pyramidal cells are characteristic of the optic tecta of many nonmammalian vertebrates. They are likely to play an important role in the tectum's vertical organization, including integration of multisensory information, because their apical dendrites extend radially, often throughout the thickness of the tectum. Therefore, we have examined turtle pyramidal cells in detail in Golgi-Kopsch preparations. Pyramidal cells are positioned with their somas in the strata griseum periventriculare (SGP) and griseum centrale (SGC). Those in the SGP form several substrata, each one cell thick. Both sets of pyramidal cells have basal dendritic fields, and apical dendrites which terminate in the stratum opticum (SO). Although the apical dendrites of SGC pyramidal cells are shorter than those of SGP cells, both pass through the strata griseum et fibrosum superficiale and intersect retinal afferents. The apical dendrites of the two types of pyramidal cells do not differ systematically, but both vary in several ways. First, some dendrites are smooth and nearly appendage-free, others have elaborate specializations, and others bear many classic spines with thin necks and round heads. Second, some dendrites do not branch at all, others branch only once, and others have three or four branches. Finally, some dendrites end in SO with round enlargements, others have complex specializations similar to those present on some dendritic shafts, and others run parallel to the pial surface for 40 or 50 μ before terminating. In conclusion, pyramidal cells in SGP and SGC both receive retinal input, but those in SGP probably receive additional inputs on their somata and the proximal parts of their apical dendrites. The existence of morphological subcategories of cells in both strata implies the existence of still undetermined functional subcategories. (Supported by PHS grants EY 05134 and NS 12518.)

1841 DISTRIBUTION OF CELLS CONTAINING CYTOPLASMIC LAMINATED BODIES (CLB) IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. M.L.Schmidt* (SPON: Helen Ghiradella). Biology Department, Union College, Schenectady, New York 12308.

The distribution of neurons containing CLBs was examined in the lateral geniculate nucleus (LGNd) and the perigeniculate nucleus (NPG) in adult cats. Formalin fixed, Paraplast embedded tissue, sectioned at 6mμ or 8mμ, was stained with Luxol fast blue and counterstained with Nissl stain. Neurons containing CLBs could be identified readily using this procedure. At present, over 10,000 cells have been counted of which approximately one third contained CLBs.

The distribution of cells in the LGNd which contained CLBs was found to be qualitatively, but not quantitatively, similar to that described previously (LeVay, S. & Ferster, D., J.Comp.Neurol. 172, 563-584, 1977): the highest proportions of cells containing CLBs were found in laminae A and A1 near the projection of the area centralis. Fewer neurons containing CLBs were found in the other layers (NIC,B) near the projection of the area centralis, however, significant numbers of cells containing CLBs were clearly present in these layers. The monocular segment of the LGNd was found to contain relatively more cells with CLBs than had been reported previously, perhaps only a few percent less than were found in the binocular segment of the LGNd. Cells containing CLBs were also present in the medial interlaminar nucleus (NIM) and in the NPG but in smaller numbers than in the laminated regions of the LGNd.

It was observed that the distribution of cells containing CLBs was not uniform in any regions of the structures that were studied: cells of this type were clustered together in groups of varying size. As a result, the variability in the proportion of cells containing CLBs is great, even between adjacent 300mμ wide segments of the same section. A precise quantitative description of the distribution of cells containing CLBs, thus, requires that large samples of cells be examined: the techniques used in this study make this a feasible undertaking.

Assuming that CLBs are present only in X-cells, then the proportions of neurons containing CLBs found in this study suggest that physiological sampling procedures are not adequate to determine the absolute proportions of different cell types. They may suffice, however, for determining relative distributions in regions of the nervous system. (Support provided by USPHS Research Grant RO1 EY-01268 to H.V.B.Hirsch)

1842 AFFERENT GEOMETRY IN THE PRIMATE VISUAL CORTEX AND THE GENERATION OF NEURONAL TRIGGER FEATURES. Eric L. Schwartz Brain Research, Dept. Psych., N.Y.U. Med. Sch. N.Y. 10016 N.Y.

The retinotopic mapping of the primate striate cortex may be mathematically represented by the complex logarithmic (conformal) mapping (1). The present work demonstrates that dendritic summation of an anisotropic cortical afferent input which recapitulates (on the scale of a cortical hypercolumn) the global retinotopic mapping, accounts for the existence of sequence regularity (1,3). Intra-cortical lateral inhibition may then flow uni-directionally, yet still create 'rotating' excitatory and inhibitory receptive field structure (3). The direction of this lateral inhibition operator is suggested to lie parallel to the boundaries of the ocular dominance slabs, (i.e. horizontally in the visual field). Since the left and right eye projections differ mainly in their horizontal component, this intra-cortical lateral inhibition would tend to enhance binocular disparity, at the cortical level. The fact that the left and right eye projections differ, in general, in their size and rotational aspects, is a serious problem for a putative role of cross -convolution in stereopsis; however, the complex logarithmic pre-processing of the visual scene provides a size and rotation invariant cortical image (1,3). This fact is discussed in the context of a spatial frequency model of stereopsis. The ocular dominance column boundaries are the approximate images, under the complex mapping log(z+1), of horizontal straight lines in the visual field. This map is regular at the fovea (z=0), is roughly linear for the central 1-2 degree's, and is essentially identical to the complex logarithm for the remainder of the field. The binocular trigger features of the primate cortex follow from the same geometrical model (3). The developmental plausability of this model is supported by noting that the 'smoothest' possible mapping of two arbitrary neural surfaces is represented by some conformal mapping which will depend entirely on the boundary conditions (shape) of the respective tissue surfaces. This is discussed in the context of the goldfish and the primate visual systems (2). The amount of genetic coding (and chemo-specificity) is minimized in this model. Finally, the fact that the local(3), global (1), and developmental (2) neurophysiology of the cortex may be succinctly described in terms of geometric function theory suggests that 'computational geometry',rather than 'neuronal feature extraction', may provide a workable paradigm for the study of visual information processing.

1. Biological Cybernetics 25: 181-194 (1977) Eric L. Schwartz
2. J. Theor. Bio. (in press, 1977) Eric L. Schwartz
3. Biological Cybernetics (submitted,1977) Eric L. Schwartz

1843 EFFECTS OF BINOCULAR DEPRIVATION ON VISUAL DISCRIMINATION. Sheila A. Scoville*, John A. Jane, and Mary D. Guthrie . Depts. of Neurosurgery and Anatomy, University of Virginia School of Medicine, Charlottesville, VA 22901.

Considerable attention has recently been given to the effects of monocular and binocular visual deprivation. This study was undertaken to determine visual behavioral deficits in binocularly deprived rats. Hooded rats were chosen for the subjects of this experiment: Three were normal (NR), and four were binocularly deprived (BDR), one of which had a visual cortex ablation. Animals were tested for performance on flux and acuity. The subjects were water deprived and were tested by means of an apparatus in which animals viewed a closed circuit T.V. monitor, and were reinforced by a spurt of water for licking a tube beneath the appropriate stimulus pattern. The major deficit observed in the BDR's was that an increased number of trials was required to achieve criterion performance. The BDR with ablated cortex required significantly more trials to achieve criterion performance than either the NR's or BDR's. The battery of more refined stimuli in the present experiments give results that expand previous results obtained with less sensitive stimulus discrimination; i.e., only black versus white and only one set of horizontal versus vertical gratings. Recent electron microscopic studies of BDR cortex which quantifies abnormal intracortical connections, and confirms earlier light microscopic results, suggest a possible anatomical basis for the deficits. At least part of the difficulty in interpretation and comparison of the behavioral results between this and previous studies lies in differences between species and testing procedures. Nevertheless, consistent interpretations relating the behavioral and anatomical evidence can be made.

1844 DIRECTIONAL SELECTIVITY OF STRIATE NEURONS IN THE SIAMESE CAT. Michael S. Shansky* and Yuzo M. Chino*. Division of Visual Science, Illinois College of Optometry, Chicago, IL 60616. W.J. Pizzi. Dept. of Psychology, Northeastern Illinois University, Chicago, IL 60625. (SPON: D.I. Hamasaki)

Directional selectivity of cortical neurons in area 17 of Siamese cats was measured and compared to similar properties in normal domestic cats. After classifying cells as simple, complex, or hypercomplex, a rectangular stimulus was positioned (using a dove prism and X/Y positioner) over the receptive field and moved at various velocities via a mirror galvanometer driven by a function generator. The direction of movement was perpendicular to the stimulus orientation, which was varied over 180 degrees. Directional preference was determined by analyzing average response histograms accumulated by a PDP-11 computer. If the peak firing rate in the non-preferred direction was less than 20% of that in the preferred direction, a unit was classified as directionally selective (Goodwin and Henry, 1976). Our data indicate a reduction of directionally selective striate Siamese units compared to normal cats. This reduction is not exclusive to either simple or complex cells alone, and may reflect the lack of Y-cells in the Siamese cat retina (Chino, Shansky, and Hamasaki, 1977).

1845 REARRANGEMENT OF GENICULOCORTICAL AND CORTICOCORTICAL CONNECTIONS IN BOSTON SIAMESE CATS. Carla J. Shatz and Simon LeVay. Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

In Siamese cats, a genetic mutation causes some fibers from the temporal half of each retina to cross erroneously in the chiasm and innervate lamina A1 of the opposite lateral geniculate nucleus (LGN). This misrouting is orderly, so that in each LGN corresponding positions in laminae A and A1 come to represent mirror-symmetric points in the contralateral and ipsilateral visual fields, but only in the contralateral eye.

How does this abnormal organization in the LGN affect the geniculocortical and intracortical connections in these animals? Previous physiological recordings from the visual cortex of the Boston variety of Siamese cat suggested that there is an orderly rearrangement in the geniculocortical projection (Hubel and Wiesel, 1971). Here, this rearrangement is examined anatomically by means of the retrograde transport of horseradish peroxidase (HRP) following an injection through a recording micropipette. In the common cat, an injection of HRP anywhere in the visual cortex labels LGN cells at a single locus in laminae A and A1. When HRP was injected at the 17-18 border in a Siamese cat, however, labeled cells were found only in lamina A1. Receptive fields recorded at the injection site were located in the ipsilateral visual field, roughly 20 degrees away from the vertical midline, indicating that this animal was a Boston cat. In another Boston cat, an injection was made at the cortical region representing the mirror-symmetric visual field position (20° into the contralateral field); labeled cells were found in LGN lamina A only. This pattern of HRP labeling confirmed the physiological results.

In view of this rearrangement, we next checked to see if intracortical connections were also rewired, by injecting tritiated proline at the 17-18 border. In 3 Boston Siamese cats, we found a projection from the border to the cortical regions within areas 17 and 18 on the same side which represent the mirror-symmetric visual field position (20° into the contralateral field). This projection was remarkable in two ways: it does not exist in the common cat, and it is functionally useless, since it links up cortical regions representing different (though mirror-symmetric) visual field positions in the same eye.

We conclude that the preservation of visual field continuity may be an important principle governing the formation of geniculocortical connections. Further, in the formation of corticocortical connections, neurons may seek out others in the same hemisphere which receive innervation from a common locus in the lateral geniculate nucleus.

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1846 SOME IPSILATERAL VISUAL CONNECTIONS IN THE ALBINO RAT. C.L.Shen* and R.H.Baisden* (SPON: C.H.Anderson) Dept. of Anat., Univ. of Illinois Medical School, Chicago, Ill. 60680.

Early investigations using silver stains to selectively impregnate degenerating boutons indicated an ipsilateral projection into nuclei of the accessory optic fiber system (Nauta and VanStraaten,1947), however, more recent anterograde studies have not confirmed projections to these centers in a variety of mammalian species (e.g. Cavalcante, et al., 1975; Giolli, 1961; Heyhow, et al., 1960; Thorpe and Herbert, 1976). As part of a project concerning the effects of unilateral enucleation on projection patterns of the remaining eye, ^{3}H-Fucose was injected into the vitreous of the rt. eye of normal controls and 1 yr. old albino rats which had the left eye removed 4 mos. prior to injection. Animals were sacrificed 1 day post-injection in order to minimize transneuronal spread of the label. Analysis of the ipsilateral projections indicated an accumulation of silver grains over the Medial Terminal Nucleus (MTN) of the accessory optic fiber system. The grain density over the ipsilateral nucleus was not as great as over the contralateral nucleus,however, it appeared to be increased in unilaterally enucleated animals as compared to controls. No evidence for ipsilateral projections to other of the accessory optic nuclei was noted. It remains to be determined if this finding is indiginous to rodents or may be generalized to other mammalian species. These results will be discussed with respect to further findings concerning ipsilateral visual connections in mature rats enucleated either neonatally or as adults.

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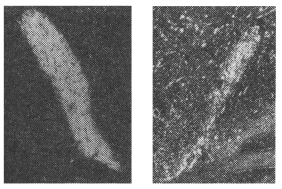
2) Giolli, J.Comp. Neur., 117 (1961), 77-95.
3) Heyhow, Webb and Jervie, J. Comp. Neur., 115 (1960) 187-215.
4) Nauta & VanStraaten, J.Anat. (Lond.) 81 (1947)127-134.
5) Thorpe & Herbert, J. Comp. Neur., 170 (1976) 295-310.



a b

Darkfield photomicrograph of retinal projection to the medial terminal nucleus in an enucleated rat. a) contralateral nucleus; b) ipsilateral nucleus.

100μ

1847 THE CAT'S PARABIGEMINAL NUCLEUS: VISUAL FIELD TOPOGRAPHY AND CELL RESPONSE PROPERTIES. Helen Sherk. Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass. 02139.

The parabigeminal nucleus, a small midbrain cell group, is known to receive a dense input from the upper layers of the cat's superior colliculus. This nucleus was studied using single unit recording techniques to determine the response properties of its cells to visual stimuli, and the topographic organization of their receptive fields.

Cells in the parabigeminal nucleus were found to respond vigorously and consistently to visual stimuli. Their properties, studied quantitatively with a computer-driven optical system, appeared to be similar to those of cells of upper collicular layers, the most notable difference being the high rate of spontaneous firing common among parabigeminal cells. The population as a whole was dominated by the contralateral eye, although, as in the superior colliculus, many cells were driven equally well by both eyes. Receptive fields were, on the average, larger than those found in superficial colliculus: within 10 deg of the area centralis the mean receptive field diameter was 2.3 deg, and for fields lying 10-20 deg from the area centralis, the mean diameter was 4.4 deg. Inhibitory surrounds similar to those of collicular neurons depressed the responses of most cells to large stimuli. The majority of parabigeminal neurons responded briskly to stationary as well as moving stimuli, and such responses tended to be quite transient. Most cells responded preferentially to a particular axis or direction of stimulus movement, and there was a tendency for the optimal axis to coincide with the meridian through the cell's receptive field and the cat's fixation point on the tangent screen.

The topography of the visual field formed by parabigeminal cell receptive fields was surprisingly orderly for so small a structure. The entire extent of the visual field represented in the superior colliculus also appeared to be represented in this nucleus. Horizontal movement from ipsilateral visual fields to the contralateral periphery coincided approximately with the rostro-caudal axis of the nucleus. Movement from upper to lower visual fields corresponded to progression from dorsolateral to ventromedial in the parabigeminal nucleus.

Preliminary recording in the pargbigeminal nucleus of the monkey (Macaca mulatta) indicates that cell response properties are similar in this animal, except that cells selective for the axis or direction of stimulus movement have not been found. An orderly map of the visual field also appears to exist in the monkey's parabigeminal nucleus.

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1848 DEOXYGLUCOSE MAPPING OF THE ORIENTATION COLUMN SYSTEM IN THE STRIATE CORTEX OF THE TREE SHREW (Tupaia glis). L.C. Skeen, A. L. Humphrey, T.T. Norton and W.C. Hall. Departments of Anatomy, Psychology and Physiology, Duke University, Durham, N.C. 27710.

Electrophysiological studies of the striate cortex in tree shrews have revealed a columnar system tuned for optimal stimulus orientation which is similar to that reported for cats and monkeys. To better visualize the topographic organization of this system, we employed an autoradiographic method for localizing stimulus-induced alterations in cerebral deoxyglucose consumption (Science, 187: 850, '75). Paralyzed tree shrews were given i.v. injections of tracer ^{14}[C]-2-deoxy-D-glucose (250μCi/kg) and monocularly exposed to a moving pattern of vertically oriented black and white stripes for 45-70 min. Their brains were subsequently processed for x-ray film autoradiography.

Autoradiographs of coronal sections contralateral to the stimulated eye revealed two patterns of increased deoxygluxose consumption in the striate cortex: 1) a continuous, horizontal strip of increased optical density restricted to layer IV, and 2) alternating radial zones of high and low optical density running through the cortical laminae perpendicular to the pial surface. The radial zones of increased optical density are approximately 150-200μm wide and probably reflect the increased activity not only of cells which responded maximally to the vertically oriented stimuli, but also that of adjacent cells having slightly different "preferred" orientations which responded at reduced rates to these stimuli. Serial reconstructions of these zones produced a map of continuous, albeit wavy, iso-orientation lines which are arranged roughly parallel to one another. They are restricted to the striate cortex and intersect its lateral border.

As suggested by previous anatomical and electrophysiological studies, these experimentally-induced radial zones of increased deoxyglucose consumption are not artifacts of monocular stimulation. They are present in identical form in the striate cortex of awake, unrestrained tree shrews which viewed vertically oriented stimuli either monocularly or binocularly, and they are not present in animals monocularly stimulated with lines of many orientations. These results reveal the topographic relationships of iso-orientation lines in the striate cortex of Tupaia. They also allow predictions concerning the organization of the stimulus orientation system which can be further examined with electrophysiological methods (see abstract by Humphrey et. al., these meetings). Supported by NSF Grants BMS 75-04230, BNS 76-18334 and NINDS Grant NS-09623.

1849 TEXTURE VIEWING, DURING DEVELOPMENT IN KITTENS, MODIFIES THE FUNCTIONAL PROPERTIES OF SINGLE CELLS IN VISUAL CORTEX. D.N. Spinelli, Dept. of Psychology and Computer Science, Univ. of Mass., Amherst, MA 01003.

Natural scenes present the visual system with information consisting of colors, edges and textures. In the cat's visual cortex Hubel and Wiesel have discovered cells that are especially sensitive to lines and edges of specific orientations. Hirsh and Spinelli have shown that if kittens are raised while viewing discordant lines with the two eyes, e.g. vertical and horizontal, binocularity of cells, in area 17, is lost and that cells with sharply tuned receptive fields have only vertically or horizontally elongated receptive fields, that can be mapped only through the eye that saw vertical or horizontal lines respectively. This powerful method can then help to answer the question of how the visual system detects textures, i.e. by first analyzing the microstructure of the texture or by processing it as an elementary feature. To this end kittens were raised in a dark room and exposed daily through their critical period for four hours, to two textures which were viewed through goggles. The two textures had identical microstructure, but appeared macroscopically quite different. The expectation was that, if the visual system first analyzes the microstructure, binocularity of cells in area 17 should not be lost and that receptive fields should be sharply tuned to the orientation of the microstructure; conversely if textures are analyzed as elementary features then binocularity should be lost and the receptive fields should not be sharply tuned. The results show that, in these kittens, cells in area 17 have lost binocularity and that the receptive fields exhibit the properties of complex receptive fields. Simple cells were not found. The implications that these findings have for visual perception and pattern recognition will be discussed.

1850 ENERGY, QUANTA AND VISION IN DROSOPHILA. William S. Stark, Robert M. Greenberg*, and Austina M. Ivanyshyn*. Department of Psychology, Johns Hopkins Univ., Baltimore, MD 21218

Drosophila eyes have 750 facets with 8 photoreceptors each. RI-6 are 6 cells/facet with peripheral rhodopsin-containing rhabdomeres. Cyclindrical rhabdomeres have packed microvilli and are ∿ 200μ by 1.5μ (diameter). Microvilli are ∿ .05μ (diameter). We calculate (1) rhodopsin optical density (OD) in vivo, (2) electroretinographic (ERG) and phototactic quantal thresholds, and (3) rhodopsin conversion adaptation intensity.

Microvilli have ∿ 4217 freeze-fracture bumps/μ^2; vitamin A deprived flies have $\frac{1}{4}$ this count (Harris, Ready, Lipson, Huspeth and Stark, Nature 266, 648, 1977). Thus, most or all bumps in controls are rhodopsin molecules. There are up to 990 molecules/microvillus=8.3×10^{7} molecules/rhabdomere=3.94×10^{-4} moles/liter. Substituting length and extinction for rhodopsin conversion at the 470nm absorption and sensitivity peak (3.3×10^{4} cm^2/mole, Harris, Stark and Walker, J. Physiol., 256, 415, 1976), OD=.26; 45% of incident light is absorbed. This density is higher than for human rods (Hecht, Schlaer and Pirenne, J. gen. Physiol., 25, 819, 1942), but rhabdomeres are longer. Other data and observations support a high OD, which causes a slight flattening of spectral sensitivity near the peak. In vitro (Harris et al.) and computed (from extinction and in vivo concentration) Δ OD's for rhodopsin conversion agree.

Drosophila ERG's are as large as 30mV. As few as 10^{10} quanta/cm^2·s or 80 quanta absorbed/rhabdomere·s elicit a 3mV receptor potential at 470nm. The absolute ERG threshold can be 2.5 log units lower for <1s, i.e., less than 1 quantum absorbed/receptor. RI-6 phototaxis thresholds are also low, about 10^{10} quanta/cm^2·s (from Schümperli, J. comp. Physiol., 86, 77, 1973). To convert half the rhodopsin with 470nm, 9.32×10^{7} incident quanta/rhabdomere =5.26×10^{15} quanta/cm^2 would be required. This agrees with physiologically assayed values (e.g., Stark and Zitzmann, J. comp. Physiol. 105, 15, 1976; Harris and Stark, J. gen. Physiol., 69, 261, 1977). Despite rhodopsin level differences, control vs. vitamin A deprived Drosophila should require similar adaptation levels for a half sensitivity decrease as was reported by Harris and Stark (1977).

Thus: rhabdomeric OD is high, ≥45%; ERG threshold is low, <1 absorbed quantum/rhabdomere; intensities ∿ 8 log units above ERG threshold elicit substantial rhodopsin conversions.

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1851 NOCICEPTIVE REPRESENTATION IN THE SUPERIOR COLLICULUS. Barry E. Stein and James Dixon* Dept. Physiology, Medical College of Virginia, Richmond, VA 23298.

Visual, somatic and acoustic representations in the hamster superior colliculus (SC) were studied with single-unit recordings. All animals (n=26) were anesthetized with urethane, and in selected experiments animals were also paralyzed with gallamine triethiodide and artificially respired. The upper layers of the SC (stratum opticum and above) contained cells activated only by visual stimuli. Deeper laminae, however, contained cells which were excited by visual, somatic or acoustic stimuli alone (unimodal), as well as individual cells which responded to more than one sensory modality (multimodal). A topographical register between visual and somatic representations was also noted.

Of the forty-six deeper laminae somatic cells studied, twenty-three responded either optimally (nonspecific nociceptor = 12/23) or solely (specific nociceptor = 11/23) to such stimuli as noxious pinch, pin-prick and/or elevation of skin temperature > 45° C. An additional twenty-four (of 39 somatic cells) cells found in adjacent tissue (subjacent tegmentum and central gray) had nociceptive properties similar to those of cells within the SC. Many nonspecific nociceptors also had low threshold mechanoreceptor properties, with the nociceptive and the low threshold receptive fields partially separated. Responses to nociceptive stimuli were blocked by etorphine (.1 mg/kg) and reinstated by naloxone (1 mg/kg) in both specific and nonspecific nociceptors. A few SC cells were also found which could be activated by both nociceptive and visual stimuli. In one cell tested, etorphine selectively blocked responses to nociceptive stimuli (responses were later reinstated by naloxone), but was without effect upon responses to visual stimuli.

The presence of nociceptive representation in the SC may account for the aversive effects of intense, yet localized, electrical stimulation of this structure in awake cats and humans. In addition, the loss of this representation may contribute to the deficits in localizing noxious stimuli which are induced by SC lesions.

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1852 ORIENTATION COLUMNS IN THE CAT'S VISUAL CORTEX M.P. Stryker, D.H. Hubel, & T.N. Wiesel Dept. Neurobiology, Harvard Medical School, Boston, Massachusetts 02115

The cells of the cat's primary visual cortex are arranged into columns according to the orientation of the bar or edge stimulus which drives the cells optimally; these columns are arranged so that preferred orientation changes gradually and progressively across the cortical surface (Hubel & Wiesel, J. Physiol. 165:559, 1963, & J. Comp. Neur. 158:267,1974). To demonstrate the shape and arrangement of the orientation columns, we used Sokoloff's histochemical method for determining local cerebral glucose uptake (Kennedy et. al., Proc. Natl. Acad. Sci. USA 73:4230,1976). One would expect when the cortex is stimulated binocularly by contours of only one orientation to see a distribution of activity which is columnar in cross section and consists of swirly or parallel bands in tangential section.

The eyes of a prarlyzed, anesthetized cat were aligned on a point in the plane of a pattern of moving vertical stripes. ^{14}C-2-Deoxyglucose was injected intravenously and visual stimulation continued for 50 to 90 minutes. Autoradiograms showed bands of label over most of areas 17 and 18. In cross section, these orientation columns were seen to extend through all layers of the cortex, with the probable exception of layer I. Density of the label was greatest, however, below and just above layer IV. Consistent with physiological differences between monkey and cat, the bands were visible in the cat's layer IVc, unlike the monkey in which this layer is labelled uniformly (W, H & S, this meeting). The mean spacing between the iso-orientation bands was 870 μm ± 70 μm SD. The pattern of these bands was irregular and sometimes discontinuous near the crest of the lateral gyrus but formed less swirled, almost parallel stripes on the medial bank of this gyrus and on the tentorial surface of area 17. In the three hemispheres examined there was a common tendency for these bands to run generally from posterior-dorsal to anterior-ventral over the greatest part of the medial bank.

1853 NEW ASPECTS OF SPATIO-TEMPORAL ORGANIZATION OF GANGLION CELLS. V. G. Sutija. U. of Miami, Miami, FL 33152.

Recordings were made extracellularly from optic tract fibers of cats with lacquer coated tungsten electrodes in a conventional set-up. Isolated units were classified into on-center and off-center, and then segregated into X and Y using linearity of spatial summation criteria. A 3 degree bipartite field of continually reversing contrast at mesopic adaptation level was used for that purpose. A 1 degree spot of light, frequency and amplitude modulated, was then positioned over the receptive field (RF) center, while the periphery received steady light of low luminance. On-center and off-center X cells responded similarly to the reversed contrast stimulus, with a pattern of responses that were mirror images of each other. Both subgroups had a null position, showing linear spatial summation, and a sustained pattern of responses at positions on either side of the null.

In the processing of time-varying stimulation, there were evident difference. Although both subgroups responded with a spike discharge that was sinusoidally modulated in response to sinusoidally modulated light, when firing rates were plotted against stimulus frequency for each modulation depth and constant average luminance, the on-center X had overall higher firing rates at all contrast levels, higher peak frequency, higher cut-off frequency and less attenuation at low frequencies, than the off-center X. The differences were statistically significant. Also off-center X had a typically higher spontaneous discharge rates at same adaptation levels and their RF centers were larger on the average at corresponding retinal locations.

The findings are consistent with recent morphological evidence (Famiglietti and Kolb, Sci., 194:193; Nelson et al., Invest.Ophthalm. 15:946) showing different anatomical pathways for on-center and off-center ganglion cells, strongly suggesting that the less direct off-center pathway is rod derived, while the more direct on-center pathway is cone derived. In cat, both, rod and cone inputs are always mixed, which makes it very difficult to separate the scotopic from photopic sensitivities. The difficulty might be resolved, if different time constants of the off- and on-center systems are also taken into account.

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1854 PHOTON COUNTING AND ENERGY DETECTION: THE EXPERIMENT OF HECHT, SHLAER, AND PIRENNE REVISITED. Malvin Carl Teich and Paul R. Prucnal*. Columbia University, New York, NY 10027.

Following the work of Hecht, Shlaer, and Pirenne in 1942, it has been widely assumed in the vision literature that the photon counting distribution of thermal light is Poisson. We indicate that the correct statistical distribution of photons is negative binomial, which reduces to the Poisson under certain conditions, such as those used by Hecht, Shlaer, and Pirenne. Under other conditions, however, the negative binomial reduces to the Bose-Einstein which is quite different in character from the Poisson.

Using signal detection theory, we obtain the optimum processor for an arbitrary photon counting distribution (a wide class of noise counting distributions will do). For a specified decision criterion (e.g., Neyman-Pearson), it is found that the simple counting processor, forming a comparison against a stored threshold count, is optimum. This is identical to the energy detection scheme proposed by McGill in 1967 for the processing of neural counts in audition. The optimum test is simple; it is the antithesis of complex information processing in which the observer is thought to abandon threshold ideas and sense likelihood ratios.

We present probability of detection curves for the specific case of generalized thermal light and show that they can differ substantially from the Poisson case. Even in the Poisson limit of the negative binomial photon counting distribution, these curves are not identical to those used by Hecht, Shlaer, and Pirenne because of the presence of noise, which manifests itself most directly in the non-zero experimental false-alarm rate. However, the curves we derive in the Poisson limit may not be distinguishable from those used by Hecht, Shlaer, and Pirenne within the accuracy of current experiments.

1855 UPTAKE OF NEUROTRANSMITTERS BY SYNAPTOSOMAL FRACTIONS FROM RETINA. Thomas, T.N., and Redburn, D.A., Dept. of Neurobiology and Anatomy, Univ. Tex. Med. Sch., Houston, Tex. 77025.

Rabbit retina were homogenized in isotonic sucrose and subjected to differential centrifugation. Low speed centrifugation (800g for 10 min) produced a P_1 fraction containing the photoreceptor cell synaptosomes (PCS). A P_2 fraction was obtained by centrifuging the resultant supernatant at 25,000g for 12 min. The P_2 fraction contained many small, conventional synaptosomes. Analytical electron microscopic techniques (C. Cotman and D. Lansburg, 1970, Br. Res. 22:152) were used to evaluate the purity of various retina fractions. The large photoreceptor cell synaptosomes (3-4 μ diameter) showed invaginations into which processes from horizontal and bipolar cells normally project. Also the PCS exhibited the characteristic synaptic ribbon.

The uptake of putative neurotransmitters dopamine, glutamic acid and aspartic acid was studied in the retinal fractions. All three transmitters showed high affinity uptake which was temperature dependent and saturable. The P_2 fraction showed the highest rate of uptake. The P_1 fraction had very little dopamine uptake, whereas the aspartic acid uptake was relatively high. Kinetic analysis of the data showed that the Km for retinal uptake is consierably lower than that of the brain.

	Km (μM)		
Fraction	Dopamine	Aspartic Acid	Glutamic Acid
Homogenate	3.35	3.36	2.52
P_1	0.15	0.94	0.51
P_2	0.96	1.70	0.86

Fractionation of retina thus yields two morphologically distinct populations of synaptosomes derived from different cell types. The fractions also showed differences in uptake patterns. Studies are underway to further characterize the uptake systems in order to identify and localize the functional neurotransmitter of the retina. Supported by USPH Grant EYO 1655-02 (DAR) and NIH Training Grant E4-07024-02 (TNT).

1856 HARDWARE SIMULATION OF CONE CELL PHOTORESPONSES. S. Vallerga*, R. Covacci*, F.W. Pottala* (SPON: M.G.F.Fuortes). NIH, Bethesda, MD, 20014.

It is well known that the vertebrate photoreceptors are connected by complex synaptic endings to both horizontal and bipolar cells, and that the horizontal and bipolar cells synapse onto one another. However, the anatomy of the retina does not reveal the direction and the polarity of the synapses. These characteristics must be inferred from the membrane potential changes recorded when the photoreceptors are stimulated by different light patterns (e.g. flashes, steps, flashes on steps, etc.).

A hardware modeling system of a limited portion of the retina could be helpful in making these inferences about the actual features of the retinal synapses.

For this purpose a hardware model of a cone cell has been designed and fabricated according to the Baylor - Hodgkin - Lamb theoretical model (J.Physiol. 242, 685-791, 1974). The model parameters have been adjusted to best fit the actual photoresponses recorded intracellularly from cones in the retina of the larval tiger salamander (Ambystoma tigrinum tigrinum, and Ambystoma tigrinum mavortium). Stimuli consisted of flashes and steps of light. The responses of the model and the real cone were matched visually and were in good agreement for 10 msec. flashes and 0.7 sec. steps of light whose intensities ranged over 2.5 log units with the intensity of the unattenuated light $1.5 * 10^{17}$ photons/sec * cm^2.

This model cone is the first step of a project involving the design and fabrication of hardware models of the retinal photoreceptor cells and second order neurons, namely the horizontal and bipolar cells. A simple network of retinal cells (10 to 12 hardware models) driven by a minicomputer should permit a fast test of all the possible synaptic connections, thus enabling us to choose the configuration more likely responsible for the observed photoresponse, and eventually design the proper electrophysiological experiment to verify it.

The project has been undertaken jointly by the Laboratory of Neurophysiology, NINCDS and the Laboratory of Applied Studies, DCRT at NIH.

1857 PLASTICITY OF PRESYNAPTIC PROTEINS IN THE RABBIT VISUAL SYSTEM. John A. Wagner*, Amy Schick Kelly* and Regis B. Kelly. Dept. Biochem., U. Cal., San Francisco, CA 94143, and Dept. Neurol., Stanford Med. Sch., Stanford, CA 94305.

We have developed a procedure that allows us to identify specific proteins in presynaptic nerve terminals, and have used it to study the presynaptic proteins appearing in the lateral geniculate nucleus (LGN) and superior colliculus (SC) of the rabbit. ^{35}Proteins were labelled by making use of axonal transport. ^{35}S-Methionine was injected into the eye. Animals were sacrificed after 36-48 hours, and the LGN, SC and optic chiasm were quickly removed and homogenized. The proteins in these tissues were then analyzed by two-dimensional gel electrophoresis. This procedure, which separates proteins on the basis of both molecular weight and isoelectric point, is capable of resolving more than a thousand protein species. Autoradiography of the gels allows labelled nerve terminal proteins to be identified even though such proteins are a small fraction of the total proteins in the regions examined. Control experiments showed that at least 90% of the radioactivity in the LGN, SC and optic chiasm is due to transported proteins.

Analysis of the two-dimensional gels from normal rabbits demonstrates that at least 160 proteins are transported to the nerve terminals in the LGN and SC. Although the majority of these proteins are transported to both, there is a small set of proteins that is transported only to the LGN and a second small set of proteins which is found only in the SC.

Visual deprivation has been shown to cause electrophysiological changes in the LGN and SC of rabbits. We sutured the eyelids of young rabbits before eye opening, and injected ^{35}S-methionine into the deprived eyes at 35 days of age. Normally open eyes of littermates were injected as controls. The results showed that visual deprivation prevents the synthesis of a single protein of the approximately 160 which are transported to both the LGN and SC of the normal rabbit. Because this protein is found in the optic chiasm of animals that have been visually deprived, we believe that this protein is a component of the nerve terminal, and not a postsynaptic protein. This protein is not present in adult animals, and thus appears to be expressed only transiently during the development of the visual system.

In summary, we have demonstrated that this type of analysis has the capability of providing an insight into the biochemical basis of the development and modification of the visual system, as well as other neural pathways. Supported by NIH grant NS09878. JAW is an NIH Postdoctoral Fellow (NS05092).

1858 A RETINOTOPIC MAP OF MOUSE EXTRASTRIATE VISUAL CORTEX. Earl Wagor, Nancy Mangini* and Alan L. Pearlman. Dept. Physiol. and Neurol., Washington Univ. Med. Sch., St. Louis, MO 63110.

Retinotopic maps of area 17 and the visual cortex surrounding it laterally and rostrally (area 18a) have been constructed for the C57BL/6J mouse using electrophysiological mapping techniques. Area 17 contains a complete representation of the visual field, with the nasal field located laterally and the upper field representation located caudally. In area 17, lines representing elevation and azimuth of the receptive field positions are everywhere approximately orthogonal, with azimuth lines running from rostromedial to caudolateral and elevation lines running from caudomedial to rostrolateral. At least two additional representations of the visual field are located in area 18a. These extrastriate visual representations are not simple mirror images of the area 17 map. In 18a, the azimuth and elevation lines are represented on the cortex in a complicated manner, and in several places are not orthogonal. The azimuth lines of 18a run roughly parallel to the line of the vertical meridian, which demarcates the lateral boundary of areas 17 and 18a. The lines of elevation, although parallel within area 17, diverge into two groups as they pass from 17 to 18a. One group consists of the lines representing elevations higher than 25° above the horizontal meridian, and the other group consists of the lower elevation lines. In area 17 the upper elevation lines are located caudally. As they pass over the 17-18a border, they curve around to head in a caudolateral direction and provide a representation of the upper part of the visual field in the most caudal 1/3 of 18a. The lines of elevation representing the lower part of the visual field are located in the more rostral and medial parts of area 17. As they pass over the 17-18a border, they turn to run in a rostromedial direction, and provide a representation of the middle and lower visual field located in the part of 18a rostral to 17. Together these two subareas provide a complete representation of the visual field. An additional complete representation of the visual field is found in the rostrolateral portion of 18a. Elevation lines located at 20° and at 30° above the horizontal meridian delineate areas of special functional significance. The cortical areas that represent the nasal visual field 20° to 30° above the horizontal meridian are expanded in both area 17 and in some regions of 18a, and one such expanded area overlaps the vertical meridian in a manner reminiscent of the expanded foveal representations found in other species. In addition, the 20° to 30° division can be effectively used to demarcate subregions of 18a, much as the horizontal meridian figures importantly in the demarcation of the boundaries of visual areas in other species. (Supported by NIH research grant EY 00621)

1859 EFFECTS OF LARGE INTEROCULAR ROTATIONAL DISPARITIES ON THE DEVELOPMENT OF BINOCULARITY IN KITTEN VISUAL CORTICAL NEURONS. Ida M. Washington*, Michael R. Isley* and Paul G. Shinkman. Univ. North Carolina, Chapel Hill, N.C. 27514.

In a recent report (Shinkman & Bruce, Science, 1977) we showed that when kittens' early visual experience consisted of left- and right-eye visual fields optically rotated in opposite directions about the visual axes (8° of rotation in each eye, giving 16° of net torsional disparity between the two eyes), the distribution of interocular differences in visual cortical cells' preferred stimulus orientations was found subsequently to be centered about the rotation experienced during early development.

The present experiment was designed as a first step towards testing the limits of plasticity in the development of interocular matching of receptive-field orientations. Awake kittens were placed in a restraining device while viewing black-white gratings that moved back and forth over the entire visual field. Each eye was exposed to a variety of grating orientations and speeds of movement, but the orientation of the gratings differed by a constant amount between the two eyes, either 45° (experimental condition) or 0° (control condition). Kittens received six 4-hr. exposures between the ages of 28-53 days. Subsequently, receptive-field organization of visual cortex was studied by conventional means.

The 45° disparity condition differed from the 0° disparity condition in three principal ways: (1) the proportion of binocular cells was significantly less in the 45° condition (58%) than in the 0° condition (79%); (2) among binocular cells, the proportion exhibiting orientation specificity in both eyes (strongly binocular) was significantly less in the 45° condition (65%) than in the 0° condition (97%); and (3) among strongly binocular cells, the variance of the distribution of interocular differences in preferred orientation was significantly greater in the 45° condition than in the 0° condition ($F = 28.24$, $p < .001$). The mean interocular difference in preferred orientation matched the kittens' experience in the 0° condition ($\bar{X} = 1.2°$) but not in the 45° condition ($\bar{X} = 13.5°$). These results are interpreted as reflecting a general disruption of binocularity produced by early visual experience in which the inputs to the two eyes have 45° of interocular rotational disparity about the visual axes.

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1860 CONNECTIONS OF THE LATERAL GENICULATE NUCLEUS (LGN) OF THE SQUIRREL MONKEY. J.T. Weber, J.H. Kaas, M.F. Huerta*, and J.K. Harting. Dept. of Anat., Univ. of Wis., Madison, 53706 and Dept. of Psych., Vanderbilt Univ., Nashville, Tenn. 37232.

The LGN of Saimiri consists of three pairs of layers, a dorsal parvocellular, a ventral magnocellular and a ventral, more poorly developed S pair. Following injections of ^{3}H-proline into one eye, label fills the dorsal parvocellular, the ventral magnocellular and the more dorsal S layer contralaterally, and the three remaining layers ipsilaterally. Label is also present bilaterally within the interlaminar zone between the magnocellular and the parvocellular layers.

Following injections of ^{3}H-proline into the superior colliculus, label is present within (1) the S layers, (2) the interlaminar zone between the two magnocellular layers, and (3) the interlaminar zone between the magnocellular and parvocellular layers.

The organization of geniculocortical projections has also been analyzed. Transsynaptic transport (TTP) studies reveal that the distribution of label is similar within both hemispheres. A dense, continuous band fills layer IV and the adjacent portion of layer IIIc, with the density being greater within layer IV. A second, much thinner band of label lies within the ventral portion of layer IIIb. Sparse label was also apparent within layers IIIa, II and I and labeled axons were present within layers V and VI. No type of ocular segregation, either vertical or horizontal in orientation, is evident in the TTP experiments.

Injections of ^{3}H-proline which involve all layers of the LGN result in a pattern of cortical labeling which differs from that present in the TTP experiments. First, label within layer IV is fine grain in appearance and evenly distributed, while that in layer IIIc appears to outline the individual fiber elements of a course plexus. Second, puffs of transported protein are present within layer IIIb immediately dorsal to the thin band within ventral IIIb. Following injections of only the parvocellular layers, label fills layer IV but not IIIc. A thin band is apparent in IIIb, but no puffs are present within the dorsal regions of IIIb. (Supported by Grants EY-1277, BMS-7500466, and NS12377).

1861 EVIDENCE FOR SELECTIVE LOSS OF X-CELL INPUT TO THE DORSAL LATERAL GENICULATE NUCLEUS OF MONKEYS WITH LONG-TERM ABLATIONS OF STRIATE CORTEX. R. E. Weller* and J. H. Kaas. Depts. Psych. and Anat., Vanderbilt Univ., Nashville, TN 37240

Retinal projections were studied autoradiographically with H^3-proline injections in two adult macaque monkeys in which most of striate cortex had been removed soon after birth. As a result of the cortical lesions, most portions of the lateral geniculate nuclei (LGN) were severely degenerated. Yet, small marginal sectors of each LGN were preserved reflecting regions of intact striate cortex. Thus, we were able to compare intact and degenerated portions of the LGN for concentrations of silver grains indicative of retinal terminations. The dense deposits of silver grains were in distinct laminar patterns. In the intact portions of the LGN, and in some of the degenerated geniculate layers, the laminar distribution of input appeared to be normal. For each magnocellular layer, label continued as an equally dense band from the intact segments across the degenerated segments of the LGN. Thus, the distribution of silver grains was not greatly affected by almost total neuronal loss. Similarly, in the degenerated portions of the LGN, silver grains reflect a normal pattern of input to a thin superficial layer, S, located between the external magnocellular layer and the optic tract. This suggests that S layer input does not depend on the presence of LGN neurons. Other input to the degenerated segments of the LGN was to the interlaminar zones. In contrast to the above regions of the LGN, the distribution of silver grains in parvocellular layers differed greatly between the intact and degenerated segments. At the junction of the parvocellular layers with the degenerated zone, the concentration of silver grains dropped off sharply. However, a thin scattering of silver grains extended the laminar pattern into the degenerated segments for a short distance. The remaining portions of the degenerated parvocellular region were free of input except for narrow concentrated bands of silver grains in some sections that may represent interlaminar input. Thus, the pattern of input to the parvocellular layers was severely disrupted by the loss of LGN neurons, while input to other regions was not. Since electrophysiological experiments indicate that X-cell retinal input is to the parvocellular layers, the results suggest a selective loss of these neurons from the retina. Indeed, examination of sections from one retina indicate a substantial loss of ganglion cells in agreement with previous studies of ganglion cell distribution after long-term cortical lesions.

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1862 UNUSUAL BIPOLAR-RECEPTOR CONTACTS IN THE GRAY SQUIRREL RETINA R.W. West. Dept. of Psychology, Memorial Univ. of Newfoundland, St. John's, Newfoundland, Canada

Previous research on mammalian retinas has shown that bipolar cells contact receptor terminals in one of two ways--as simple "flat" contacts which have little junctional specialization other than a prominent presynaptic thickening or as participants in a synaptic complex which includes a ribbon-like organelle in the receptor cytoplasm. Golgi-EM of the cat and primate retinas has shown that the bipolars make exclusively flat or ribbon-related contacts only onto cones or exclusively ribbon-related contacts only onto rods.

Golgi-EM of the gray squirrel retina now shows that this pattern is not universal among the mammals. While the gray squirrel bipolar cells also make flat and ribbon-related receptor contacts, two out of five bipolar subtypes do so in an unusual way. One bipolar cell subtype, which contacts rods exclusively, simultaneously makes flat as well as ribbon-related junctions. The unusual flat junctions may be identical to those previously described in the squirrel retinas as prominent presynaptic thickenings on rod terminals. A second unusual bipolar subtype, which makes flat contacts onto cones, makes a small number of flat contacts onto rods as well. The presence of these unusual bipolar-receptor contacts may be related to the many cone-like morphological and physiological features which characterize the squirrel rods.

1863 ORIENTATION COLUMNS IN MONKEY STRIATE CORTEX AND THEIR RELATIONSHIP TO OCULAR DOMINANCE COLUMNS T.N. Wiesel, D.H. Hubel, and M.P. Stryker. Dept. Neurobiology, Harvard Medical School, Boston, Massachusetts 02115.

The ^{14}C 2 deoxyglucose method recently developed by Sokoloff and his group (C. Kennedy et al., Proc. Natl. Acad. Sci. USA. 73: 4230, 1976) was used to visualize orientation columns in the macaque monkey striate cortex, following stimulation with vertical lines. Two weeks before the experiment we injected one eye with ^3H-proline to label by transneuronal transport the geniculate terminals in the ocular dominance columns corresponding to the injected eye (Wiesel, Hubel and Lam, Brain Res. 79:273, 1974). At the time of the experiment we stimulated the anesthetized monkey for 45 minutes after the deoxyglucose injection, moving back and forth a large screen consisting of vertical white stripes on a black background. Both eyes were open and aligned with a prism so as to superimpose the foveal projections on the screen. The ^{14}C autoradiographs of striate cortex showed vertical bands of label extending through the full cortical thickness, except possibly for layer I, which was only faintly labelled. Label was most intense in layers IV b (line of Gennari) and VI. Layer IV c was labelled uniformly throughout striate cortex, as expected given the lack of orientation specificity of units recorded in this layer. The pattern seen in tangential sections was complex, consisting of swirling stripes with many bifurcations and blind endings, but with occasional more regular regions of roughly parallel stripes. Interstripe distance was rather constant at 570 μm. Eye dominance stripes in layer IV c from the eye injection were revealed by washing the fixed sections for some hours in water to remove the deoxyglucose, dipping them in emulsion, and exposing them in the dark for several months. These stripes had a period of 770 μm and were simpler in their pattern, with fewer bifurcations and swirls. A comparison of the two sets of columns in the same area showed extensive intersections, without however any strict or consistent relationship: angles of intersection were distributed as expected for any two randomly superimposed sets of lines. Tangential sections from a second monkey stimulated with vertical stripes, with only one eye open, and injected with deoxyglucose showed regular rows of patches except in IV c, where the rows were uniformly stained. Again this pattern of labelling is predicted from, and confirms, the physiological studies.

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1864 MONOCULAR AND BINOCULAR PARALYSIS PRODUCE DIFFERING PATTERNS OF CHANGE IN THE LATERAL GENICULATE NUCLEUS OF THE ADULT CAT. P. R. Wilkerson*, W.L. Salinger, and M.G. MacAvoy* (SPON: M.R. Harter). Psych. Dept., Univ. N. Carolina, Greensboro, 27412.

Data collected in cortex of adult cats (Maffei and Fiorentini, Brain Research, 1976) indicate that binocular paralysis (abnormal and symmetric loss of ocular motility) has no effect on ocular dominance distributions of cortical cells. Monocular paralysis (abnormal and asymmetric loss of ocular motility), however, produces a clear loss of binocularity in the visual cells of the cortex. The study reported here was conducted to determine if the effects of monocular and binocular paralysis on cortex are mirrored in the electrophysiology of the lateral geniculate nucleus (LGN) of the adult cat.

LGN is known to undergo substantial electrophysiological change following monocular paralysis (Salinger, Schwartz, and Wilkerson, Brain Research, 1977). In contrast to reports on visual cortex, changes in LGN following binocular paralysis were in evidence as well. Binocular paralysis, like monocular paralysis, induced selective losses, indicated by decreases in the relative frequency of encountering LGN cells with response latencies to optic chiasm shock (OX latencies) in the X-cell range. These changes were, however, different from those seen in the LGN following monocular paralysis. The losses induced by binocular paralysis occur more quickly than is the case following monocular paralysis. Moreover, cell losses produced by binocular paralysis were relatively more uniformly distributed across LGN laminae than was the case following monocular paralysis. With monocular paralysis, the losses were more concentrated in, but not limited to, the lamina served by the mobile eye.

In the LGN it appears that abnormal oculomotor innervation in the adult, with or without asymmetry of motility, is effective in altering the normal organization; while according to Maffei and Fiorentini, in cortex, only asymmetrically abnormal innervation is sufficient to alter the normal organization. We have no explanation for the apparent failure of LGN and visual cortex to respond to binocular paralysis in a parallel fashion.

1865 CONNECTIONS BETWEEN THE DORSAL LATERAL GENICULATE NUCLEUS AND VISUAL CORTEX IN THE MACAQUE AND SQUIRREL MONKEY. J.R. Wilson, A.E. Hendrickson, and M.P. Ogren. Dept. of Ophthalmology, University of Washington, Seattle, Washington 98195.

Projections between the dorsal lateral geniculate nucleus (dLGN) and visual cortex were studied in Macaque and squirrel monkeys using the techniques of orthograde and retrograde axonal transport by either a direct injection of a mixture of radioactively labeled amino acids and horseradish peroxidase (HRP) into the dLGN or injections of H^3 proline and fucose into one eye. We found no convincing evidence for any connections between the dLGN and prestriate area 18 in Macaque or squirrel monkey by either experimental method. In all cases radioactive label in area 17 declined abruptly at the 17/18 border to remain at background levels throughout area 18. We also looked closely in area 18 for HRP-labeled neurons but only a few scattered cells were found.

In contrast to area 18, a large number of neurons in layer VI of area 17 were labeled with HRP. Direct injection of parvocellular layers resulted in autoradiographic labeling of terminals in layer IVCβ and IVA while labeled magnocellular terminals were found only in IVCα. Large injections involving several layers of the dLGN of Macaques frequently showed regularly repeating autoradiographic labeled/non-labeled patches within layer IVC resembling the ocular dominance columns reported by Hubel, Weisel and co-workers. Within each patch, layer IVCα and/or IVCβ were homogeneously labeled while layer IVA often had 2 or 3 small, intense areas of label. Squirrel monkeys had homogeneous labeling in layers IVA and IVC with no sign of any banding patterns. In some animals, layer VI also appeared lightly labeled. One Macaque showed orthograde transport of HRP from the dLGN into layer IVC of area 17 which again formed regularly repeating patches. HRP positive neurons were found in layer VI under these labeled patches suggesting that the cortical feedback is to the same layer(s) of dLGN which are projecting to the patches of layer IV; i.e., a geniculo-cortical loop dominated by one eye.

Our results show that monkeys, unlike cats, have no direct dLGN input to area 18. There is also additional separation of inputs from the dLGN to layer IV of monkeys which is not seen in cats. Finally, there appears to be a basic difference in organization between the visual geniculo-cortical pathways of Old and New World monkeys; Old World monkeys have a separation of the ocular inputs within layer IV of 17, while New World monkeys have a mixed input. (Supported by grants EY01208, EY39039, and EY07013)

1866 PERFORMANCE OF 4-CHOICE BRIGHTNESS DISCRIMINATIONS BY CATS WITH LESIONS OF THE SUPERIOR COLLICULUS. J.M.S. Winterkorn and T.H. Meikle, Jr. Anatomy Dept., Cornell U. Med. Coll., N.Y., N.Y. 10021

The superior colliculus (SC) has been implicated in several aspects of attention. Goodale and Murison (Brain Res.88:243,1975) have reported that rats with lesions of the SC follow more direct paths to the correct stimulus than intact rats in a multiple choice brightness discrimination and are less distracted by novel stimuli than intact rats. They concluded that deficits in attention following ablation of the SC are reflected in decreased distractibility.

In the present experiment, cats were trained for food reward in a visual discrimination, in which 4 stimulus panels were presented along one wall of a 6-ft. square open field. The path of approach to the correct one of the 4 stimulus panels was recorded for each cat (a) when learning a brightness discrimination and (b) when presented with a distracting visual stimulus after the cat had learned the discrimination to a high performance criterion. Four cats were trained and tested before and after bilateral ablation of the SC and 3 cats were trained and tested only postoperatively.

All intact cats and cats with lesions of the SC learned the 4-choice brightness discrimination to the same high levels of performance. Contrary to Goodale and Murison's results with rats, cats with lesions of the SC followed longer paths in approaching the correct stimulus panel even after attaining criterional performance. In addition, both intact cats and cats with lesions of the SC were distracted by novel stimuli, but both groups quickly habituated to the distracting stimuli. These results do not support the hypothesis that lesions of the SC produce deficits in attention as measured by distractibility to novel stimuli.

1867 SYNAPTOGENESIS IN THE OUTER PLEXIFORM LAYER OF THE RETINA OF THE CLAWED TOAD XENOPUS LAEVIS. Paul Witkovsky and Frank D.H. Chen* Dept. Anat. Sci., SUNY Stony Brook, N.Y. 11794

The initial appearance and subsequent development of synapses in the outer plexiform layer of the Xenopus tadpole have been examined by electron microscopy. Photoreceptor synaptic ribbons with a halo of agranular vesicles first appear at Nieukoop-Faber stage 37/8 but at this stage often are not aligned with postsynaptic processes. E-PTA staining revealed that the arciform density and adjacent membrane specializations of post-synaptic profiles were barely visible at stage 37/8, thereafter increasing in both density and extent. Serial section reconstruction established that the post-synaptic element associated with the photoreceptor synaptic ribbon was invariably a horizontal cell (HC) dendrite. A few en passant ribbon synapses made onto HC dendrites at the base of the photoreceptor cell were noted, but the main synaptic input to the HC was within the receptoral invagination where 4-9 ribbons made contact with the several lobules derived from a single HC dendrite. Additional HC membrane specializations away from the ribbon were noted.

Bipolar cell (BC) dendrites made only basal junctions with the photoreceptor in the tadpole stages 37/8 - 46 studied. The basal contact was characterized by a wide synaptic cleft containing transverse bars. With E-PTA staining, basal junctions were trilaminar, the photoreceptor paramembranous material appearing somewhat denser than that of the BC dendrite. The third line of electron-dense material was located in the synaptic cleft. Although BC basal junctions were first noted at stage 37/8, their numbers increased markedly after stage 42.

No conventional synapses were found in the outer plexiform layer of the retina in stages 37/8 - 46, but they were encountered in a late-stage, pre-metamorphic tadpole.

The data indicate (a) that the morphological substrate of photoreceptor to HC, and photoreceptor to BC, transmission differ substantially, and (b) that there is a sequential appearance of specific synapses in the retinal outer plexiform layer during tadpole development.

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1868 3',5'-GUANOSINE MONOPHOSPHATE AND THE IN VITRO PHYSIOLOGY OF FROG PHOTORECEPTOR MEMBRANES. Michael L. Woodruff and Deric Bownds. Dept. of Zoology and Laboratory of Molecular Biology, Univ. of Wisconsin, Madison, Wisconsin 53706.

A cyclic nucleotide system may be important in photoreceptor function. It is now well established that 3',5'-guanosine monophosphate (cGMP) is found at high levels in dark-adapted retinal rod outer segments (ROS), and that illumination decreases the cGMP concentration. The experiments reported here measured directly (by radioimmunoassay) the levels of cGMP in freshly isolated frog ROS to establish light sensitivity, kinetics and correlations with plasma membrane permeability. The data suggest that cGMP may mediate light-induced permeability changes.

Illumination can decrease the concentration of cGMP from approximately .015 moles cGMP/mole rhodopsin to .007 moles cGMP/mole rhodopsin. The light-dependent cGMP decrease is rapid and reversible. A significant decrease is observed 200-400 ms after the onset of illumination, and after brief light exposures the concentration returns to near the dark value within 60 seconds. The decrease on illumination represents a substantial amplification. The capture of a single photon can reduce the cGMP level by 2×10^4 molecules.

Light suppression of ROS permeability and the light-dependent cGMP decrease become larger as illumination is increased and vary with the logarithm of light intensity at levels which bleach between 5×10^2 and 5×10^5 rhodopsin molecules/outer segment-second. The correlation between cGMP and permeability is maintained through three pharmacological perturbations. The phosphodiesterase inhibitor papaverine increases both cGMP levels and permeability; β,γ-methylene adenosine triphosphate or calcium increases the effectiveness of light in reducing both cGMP and permeability.

1869 MEMBRANE CURRENT RECORDING FROM SINGLE ROD OUTER SEGMENTS. King-Wai Yau*, Trevor D. Lamb and Denis A Baylor. Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305

Vertebrate photoreceptors respond to light with a reduction in the steady sodium current which in darkness flows inward across the membrane of the outer segment. We have developed a method for recording membrane current across single rod outer segments in the toad retina. The method was to draw an outer segment projecting from a piece of isolated retina into a close-fitting glass suction electrode filled with Ringer solution and connected to a current-to-voltage transducer. Flashes of visible light evoked slow outward membrane current with properties generally similar to voltage responses recorded with an intracellular electrode; the initial spike on the voltage response to bright flashes has, however, not been seen in the current recordings. The maximum photocurrent obtainable from a cell corresponded closely to a complete suppression of its inward dark current. Dim light evoked fluctuations in the membrane current which were apparently composed of single photon effects having peak amplitudes of about 0.5 pA and a slow time course similar to responses to brighter flashes. The smooth form of the elementary photon effects suggests that many ion channels or carriers may be blocked by one photoisomerization. A dark current noise suppressed by bright light has also been observed. This dark noise is composed of faster elementary events than the photon noise.

1870 SPECTRAL OPPONENCY AND ASYMMETRY BETWEEN CONE MECHANISMS IN THE CAT ELECTRORETINOGRAM (ERG). Eberhart Zrenner* and Peter Gouras. National Eye Institute, NIH, Bethesda, Md. 20014

In the d.c.-ERG of the isolated perfused cat eye two cone mechanisms (λmax=450 and 555nm) can be identified by their action spectra obtained by constant response criteria with monochromatic Ganzfeld-stimuli in the presence of intense chromatic adaptation (12 experiments).

At the onset of the light stimulus and in the presence of strong yellow adaptation both cone mechanisms appear to sum their contributions to the negative on-response (P III) producing a broad, flat single peaked action spectrum, fitting a summation of 450 and 555nm Dartnall-nomograms when corrected for lens absorption. Under the same conditions they appear to oppose each other in their contribution to the positive on-response (b-wave) producing a double peaked action spectrum (peaks near 450 and 555nm) with a large sensitivity loss near 500nm, fitting a subtraction of both nomograms. This double peaked function cannot be due to interaction between a 555nm cone mechanism and rods because the short wavelength branch remains unaltered over a 16-fold increase in yellow adaptation, whereas the long wavelength branch follows the Weber-Fechner law.

When isolated, the sigmoidal intensity-response function for the 450nm-mechanism has a lower slope and saturates at several μV maximum amplitude; that of the 555nm-mechanism is steeper and cannot be saturated with our maximum intensity. The intensity response function for the former could be made to approximate the latter by multiplying it by a factor of at least 10, possibly the ratio of 555 to 450nm cones.

At the offset of the light, both cone mechanisms generate a slow negative response but only the 555nm-mechanism produces a quick positive response also. The action spectrum of the negative off-response resembles that of the negative on-response (P III), whereas the action spectrum of the positive off-response shows no participation of the 450nm-mechanism at all. We interpret this on-off assymmetry to mean that the positive off-response is generated by a quick return of the 555nm cone receptor potential, not present in the 450nm cones.

We conclude that there are at least two cone types in cat retina: One more numerous, more long wavelength sensitive and more rapid in its response; the other less numerous, more short wavelength sensitive and slower in its time course resembling in some respects rods. At a point beyond the receptor level (P III) but before (or at) the site of b-wave generation opponency between these two cone mechanisms seems to occur.

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